

nature

May 29, 1975

He wants your opinion

IT has been a busy week for Lord Crowther-Hunt, Minister for Higher Education at the Department of Education and Science, who has delivered three major speeches on the subject of priorities within the higher-education sector. These, coupled with Mr Prentice's recent appearance before a House of Commons Select Committee, go some way towards reassuring those in higher education that the department, which has kept a distinctly low profile in this field for a long time, has not forgotten about them entirely. And the generally thoughtful nature of these contributions, positively inviting dialogue, deserves an intelligent response from the intellectual community and from employers.

The problem confronting educational planners is this. At present there are about 460,000 full-time students in universities and in polytechnics and colleges in England and Wales. There is still a commitment to raise this figure to 640,000 by 1981. But this sort of expansion could not be carried out against a background of economic stagnation unless the government were to devote what growth money it had to higher education, to the detriment of schools, hospitals and so on. This it clearly will not do; Lord Crowther-Hunt does at least go through the motions of musing whether there is a strong relationship between investment in higher education and long term economic growth, but concludes only that "it would be asking too much for a reasonable man to believe that a higher quality labour force does not have a higher potential than a lower quality one, while recognising at the same time that quality in this context has many dimensions". Hardly a strong case for Mr Prentice to argue in Cabinet for preferential treatment for higher education to get us out of our economic hole. Thus the recent Budget cuts of £1,000 million in public expenditure planned for 1976/7 mean substantial cuts in education and science, and instead of a growth rate of 2.7% in that year, there will only be room for about 1% more.

Inevitably, one must then ask whether expansion to 640,000 makes sense, and if it does, what sort of education this means for the swollen numbers. The answer to the first question is almost certainly that the target will not be reached, although obviously the government would like not to have to be responsible for taking that decision itself; it may well be let off the hook by the continuation of the trend amongst the young to forego higher education voluntarily. But even if targets are lowered, it is inescapable that the atmosphere within the higher education world will have to change. Lord Crowther-Hunt dropped several hints of things to come:

- Student/staff ratios are bound to rise from their present value in universities of 8.4 : 1. A figure of 10 : 1 or even 11 : 1 was mentioned—from which it could be inferred that present staffs would spend more time teaching, rather than that new staff would be recruited.

- Manpower planning will be conducted in an attempt to respond to the perceived future needs of employers.

- Efforts will be made to make courses more relevant to the needs of the country.

- Universities and polytechnics will be urged to collaborate more.

- The balance between teaching and research will have to be looked at.

Some of this can be welcomed, particularly any efforts within the manpower planning field to understand how the young decide what to study—and whether they are open to persuasion to change their mind. The worry must always be whether the planners' crystal balls perform any better than randomly in predicting needs five or ten years hence. Any positive intervention at present would presumably be devoted to restocking chemistry, and to a lesser extent, physics departments; it would be unlikely to be urging yet more students to think of medicine as a career. Yet how much confidence can we really have that by responding to present imbalances we shall create future prosperity? Maybe the chemical industry would profit more from an influx of mathematicians and the medically-trained than by continuous recruitment of chemists, and maybe this is unknowable before the fact.

Similar concern must also be expressed that 'relevance to the nation's needs' can be pushed much further without producing dreary courses of study away from which bright students will flee. Lord Crowther-Hunt's contribution to the debate here is less than worthy—"many educationists will disagree [with relevance] but they are surely going to find it very difficult indeed to argue for the contrary principle, namely 'irrelevance'". Many besides educationists will find such stark categorisation unhelpful. The contrary principle to which many devote their teaching career is intellectual agility and flexibility and is surely as much to be sought after now as ever.

There has as yet been scarcely a murmur from the academic community at the increase in productivity called for if student/staff ratios are to rise. But there is one quite serious problem which deserves early airing. The higher education world is not like some vast nineteenth-century construction industry where the foreman/navvy ratio could be juggled at will by hiring and firing. For better or worse almost all staff have tenure. Further, staff tend to come in thousands of little cells called departments, each with its own proud independence. A smooth change in student/staff ratio at the macroscopic level can produce some hideous anomalies at the microscopic level.

Lord Crowther-Hunt has done the higher-education community a service by giving early public notice of change. The community can help mould its future by an intelligent response. □



A headache for US industry

On April 28, 1971, the Occupational Safety and Health Act came into effect in the United States, establishing a new agency—the Occupational Safety and Health Administration (OSHA) in the Department of Labor—with a mandate to enforce health and safety standards for America's 60 million workers. As expected, the act has turned out to be a huge headache to enforce and administer, not only because of the enormous costs to industry in altering its technology and work practices to comply with federal standards, but also because the act does not diminish, but raises, discontent about conditions in the workplace. It exposes the sores of industrial practices and labour union conservatism and raises the profound question of what research in occupational health should be directed towards. Wil Lepkowski reports.

NEXT month, OSHA will begin a series of public hearings on its proposed noise standard of 90 decibels which will be both long and costly. Dow Chemical Corporation alone has asked for five days to present its case against that proposal. Consumer and labour groups, on the other hand, will argue for the figure of 85 dB being pressed for by the Environmental Protection Agency (EPA). (The EPA's standards inevitably are more stringent than OSHA's, on the unspoken assumption that the workplace is inherently more dangerous than the outdoors).

The case against noise will be argued in the context of hearing loss, but another aspect is creeping into the acoustic consciousness: frequency. Psychophysicists are finding that various frequencies can exert subtle but profound effects on the human brain and nervous system. If the 90dB level is adopted (and the EPA will fight its sister agency OSHA for the 85 dB standard to be established), it will cost industry \$30,000 million to comply, according to a study by the engineering consultant company Bolt, Beranek and Newman. If research is able to correlate frequencies with, say, the accident rate or the vague condition known as *ennui* then occupational safety and

health could reach another level of complication involving design of machinery and the mind-body-environment interaction in the workplace.

Consider stress, for example. The research arm of OSHA, the National Institute of Occupational Safety and Health (NIOSH), recently published proceedings of a conference on behavioural toxicology which raised questions about the role of stress in job safety, health and performance. OSHA is nowhere near developing standards for reducing stress on the job—unless one regards noise and heat as stress inducers. But OSHA's standards director Daniel Boyd agrees that stress is an obvious problem in the workplace which is related to development of chronic disease, and that standards will eventually govern work practices that produce stress. The question, however, is how one separates workplace stress from stresses of the home, family, and environment. At what point does occupational health spill over into community health?

Cancer is often considered to be a disease induced in the workplace, but heart disease is not. Why? Because carcinogens are known to be present in the workplace, whereas non-psychological causes of heart disease are harder to spot. Thus, heart disease is considered a community health problem, whereas cancer is increasingly looked on as a job-related disease.

The new NIOSH Director, John Finklea, will have plenty of housework to do before he ventures out with his first indictment. The agency's entire research programme is ripe for reorganisation, for example. "There's no direction to the research they do there," declares Nicholas Ashford who is completing a large study of the occupational safety and health for the Massachusetts Institute of Technology Center for Policy Alternatives. "There are no deliberate attempts to formulate any kind of research policy based on real problems in the workplace," he says. "They don't bring input from labour into the design of their research projects. What they have is a lot of old-time researchers who are accustomed to pursuing their own interests and going their own way without regard to the timeliness of their work"

Sheldon Samuels, director of health, safety, and environmental affairs for the Industrial Union Department of the AFL-CIO, is even harsher. "They have a lot of dead wood and meaningless programmes there," he says. "Their hazard evaluation programme is a total failure. The evaluations they do are meaningless. They don't really evaluate present and future hazards. All they do is measure how close companies come to the threshold limit values. And when they get important

information they sit on it without alerting anyone."

Finklea is keenly aware of the criticisms. One of his plans is to reduce by 10% all newly induced cancers in the workplace. The strategy is unclear and, as the forward plan for NIOSH states, "the full impact may not be evident for approximately 20 years because of the disease latency period". What will certainly be involved is coordination with several agencies that deal with carcinogenesis, as well as a much stepped up cancer epidemiology programme. For example, the National Center for Health Statistics has no data on disease broken down by occupation. That will have to change.

Just as rising water finds every nook and cranny in a tidal cave, so OSHA extends into every occupation in the United States. In the operating theatre, studies have shown that anaesthetic gases are the cause of birth defects in the children of nurses exposed to those gases and even in the children of male physicians similarly exposed. OSHA is satisfied, however, that the medical profession is doing an adequate job of regulating its own operating room environment now that the danger is known.

It is even conceivable that OSHA could regulate the methodology of genetic research if the scientists themselves do not succeed in drafting a set of procedures that prevent the spread of potentially hazardous organisms into the atmosphere. The subject was raised by a Washington lawyer, Harold Green, during the meeting on genetic transplantation organised by the National Academy of Sciences at Asilomar, California.

A lawyer from the University of Indiana, Roger Dworkin, warned that if scientists did not regulate that research, OSHA could move in and do it for them.

The individual most responsible for the direction that occupational health research takes at a national level is John Finklea. A South Carolina paediatrician, he has a penchant for controversy as well as skill at diplomacy and could be a much-needed antidote to his predecessor Marcus Key, who preferred diplomacy at all times. As head of the National Environmental Research Center under the EPA, Finklea was in the thick of the debate over sulphate pollution from power-plant chimneys and from the catalytic converters in automobiles. Suffice it to say that Finklea's position as an opponent of tall chimneys and the catalytic converter hastened his departure from the EPA.

Finklea has the backing of both environmental and labour groups, but industry is cautious. "It's true Finklea doesn't shy away from controversy,"

says Kenneth Nelson, vice-president for environmental affairs at American Smelting and Refining Company, which was stung by NIOSH's recommendation of a no detectable limit standard for arsenic. OSHA went along with the recommendation and issued it as a proposed standard in March. "His pronouncements on sulphates at EPA were quite devastating. There's absolutely no evidence that they are dangerous in environmental concentrations. But suddenly his position became gospel. I don't want to prejudice him, but this is the kind of thing we don't want to see him do at NIOSH."

The most scaring thing NIOSH deals with is the fear that an 'epidemic' of industrial cancer may be beginning which is several years away from hitting with full force. There's no apparent way to prevent such an event from happening once the cancer process has begun. "We're talking about problems we will have 60 years from now," says Finklea. "That is something we've never faced in NIOSH."

The vinyl chloride episode stunned industry into recognising some of the more frightening consequences of exposure to toxic substances. It was also a huge embarrassment to all the government agencies which oversee environmental health. NIOSH was shocked and embarrassed, but not as acutely as the National Cancer Institute (NCI). NIOSH, after all, could plead scarcity of funds and manpower with its overall budget of \$700 million. The NCI had no such let-out. Critics said correctly that it was guilty of misplaced priorities. Now it is trying to catch up.

Since that episode, the institute has stepped up its cancer bioassay programme to intensify its relevance to occupational exposure to carcinogens. It now issues so-called 'cancer alerts' to an inter-agency panel whenever environmental and epidemiological researchers at the National Cancer Institute come across data showing links between chemicals in production and the cancer process. The latest such alert was issued for trichloroethylene, triggering action by NIOSH's hazard surveillance teams to assess the extent of the problem throughout industry.

The NCI knows it needs much more information about carcinogenesis on the personal level—that is, on the level at which the work environment affects cancer susceptibility and, in fact, prevention. Its newly emphasised cancer control programme will begin studies on cancer control in the workplace. And the environmental carcinogenesis programme under Dr Herman Kraybill, is gradually working out written agreements with companies that will give NCI researchers access to the medical workers exposed to chemicals suspected of being carcinogenic.



Finklea: not afraid of controversy

Industry itself has made a quantum leap into responsibility, not only by opening up its records—unprecedented in the past—and broadening its medical examination of workers to a score of metabolic parameters but also through formation of an inter-company toxicology research programme to give early warning to companies about impending problems. The project is new and requires the working out of some legal difficulties over transfer of what once was considered privileged information. But now worker health is taking precedence over industrial secrecy.

"What we're seeing now," says Kraybill, "is industry moving slowly towards closed systems in its chemical production processes. The big companies can do this. But it's the smaller companies that are causing many of the problems". The small companies do indeed present difficulties. In most cases they exist in small towns where the work force is small and has few other job opportunities. Profits are not substantial, and work and production practices are sloppy. In one rural town in Maryland a small company was found to be poisoning the entire community through the dumping of chemical wastes in an adjoining stream. Research offers no solution to this kind of situation. And even now, it has not been possible to find the customary one-to-one ratio between the offending agent and the mortality in that Maryland village.

Although occupational health researchers see no broadly encompassing methodology that would substitute for the chemical by chemical assessment of

health hazards, they are agreed that a single standard test protocol for bioassay can be developed. The process is somewhat akin to working out an international treaty: a text is drafted and re-drafted until each faction makes the compromises necessary for agreement. The National Cancer Institute is being looked on by the toxicology profession as a high arbiter in the resolution of controversy. It is not a regulatory agency but is the focus for most frontier regions in cancer research and so can be depended on to harness the information needed to present a consensus.

Finklea realises the need for a generalised research theory of occupational health but sees none on the horizon. What he would like to see at the very least is NIOSH run as a single 'system', flowing from workplace problems, to applied and basic research needed to understand it, to the production of a criteria document or OSHA recommendation. As such, he is as much directing policy research as a series of projects. Each criteria document alone now costs close to \$1 million in contractor costs, far too high and counter-productive. And OSHA very often rejects the recommendations set out in them. In the science policy sense, NIOSH, by focusing just on the human problem, does seem to synthesise in one place the many aspects contained in Francis Bacon's dictum to scientists: "... that they consider what are the true ends of knowledge, and that they seek it not either for pleasure of mind, or for contention, or for superiority to others ... but for the benefit and use of life; and that they perfect and govern it in charity." □

international news

UNLIKE radioactive substances, and a few animal pathogens such as foot and mouth virus, work on pathogenic micro-organisms in the United Kingdom has been subject to few statutory controls. Laboratories working with many viruses and bacteria pathogenic to humans and animals are not required to register their work and such codes of practice that do exist and are followed are voluntary and drawn up by the workers themselves in self-defence.

But opportunities for work with 70 pathogenic bacteria, viruses and other microorganisms will be severely curtailed in the future, according to a report published last week by a working party under the chairmanship of George Godber (former Chief Medical Officer of Health at the Department of Health and Social Security, DHSS) which was set up to investigate the laboratory use of dangerous pathogens following the escape of smallpox virus from the London School of Hygiene and Tropical Medicine in 1973.

The first task of the working party was to draw up a list of laboratories working on a check list of 66 pathogens considered particularly dangerous. A questionnaire sent to all biological laboratories in the country, including

Report wants controls on bacteria work

those in universities, hospitals, the public health service and industry, identified 595 laboratories holding one or more of the pathogens listed. This says the report is a much larger number than expected.

The most widely held organism was *Salmonella typhi* (held by 322 laboratories) followed by *Mycobacterium tuberculosis* (289 laboratories), *Vibrio cholerae* (194 laboratories) and *Brucella* (179 laboratories). The working party was particularly concerned that *S. typhi* was one of the organisms used for routine testing of disinfectants and recommends that the relevant British Standard be amended to permit another suitable organism such as *Escherichia coli* to be used. It also recommended that the practice of using pathogenic strains of *M. tuberculosis* for teaching should be discontinued.

Its main recommendations were that 30 of the most dangerous organisms

(including herpes B virus, Lassa fever virus, foot and mouth virus, rabies virus and smallpox virus) should be designated category A organisms and should only be studied under the most stringent conditions. Control of their possession and use should be exercised by some central body. Category B organisms (including many of the human and animal bacterial pathogens) should only be studied in laboratories under the control of suitably qualified staff following codes of practice yet to be drawn up but similar in essence to guidelines contained in *Safety in Pathology Laboratories* and *The Prevention of Laboratory Acquired Infection* (DHSS).

The recommendations regarding category A pathogens involve the establishment of a Dangerous Pathogens Advisory Group (to be chaired by Professor R. A. Shooter) which would advise on the licensing of individual laboratories and conditions under which work should be done. Although this system would be voluntary at first, it will eventually require statutory controls. Complete confidentiality would be maintained. The import of category A pathogens into the country should also be strictly controlled and should only be allowed when the work to be done is of "overriding importance". Detailed recommendations for dealing with category A pathogens are given in the report. Implementations of these recommendations will require in most cases completely new laboratory buildings.

● That the total number of laboratories holding pathogens in categories A and B should be reduced by ceasing the practice in any laboratory which cannot show good reason for continuing it.

● When work involves any special risk to the public a decision on whether work should proceed should be made outside the laboratory.

● General guidance on safety in laboratories should be drawn up and applied.

● International standards for the control of dangerous pathogens should be drawn up.

● No pathogen should be handled in a laboratory except under the supervision of suitably qualified staff.

● The categorisation of dangerous pathogens included in the report should be kept under review and modified as necessary in the light of future discussions and experience.

● A safety officer should be appointed in any laboratory holding dangerous pathogens. □

Category A Pathogens	No of establishments		No of establishments
Herpes B virus ...	4	Bat rhabdovirus ...	6
Lassa Fever virus ...	1	Pustular Dermatitis virus ...	24
Marburg virus ...	1	Duck Plague virus ...	7
Rabies virus ...	13	Equine Anaemia virus ...	2
Smallpox virus ...	19	Equine Arteritis virus ...	1
African Horsesickness virus ...	1	Bovine Rhinotracheitis virus ...	24
African Swine Fever virus ...	3	Kyanasur Forest Disease virus ...	3
Bluetongue virus ...	1	Murine rhabdovirus ...	1
Equine Encephalomyelitis group ...	29	Serum Hepatitis virus ...	98
Foot & Mouth Disease virus ...	2	Gastroenteritis virus (pigs) ...	11
Fowl Plague viruses ...	24	Yellow Fever virus ...	3
Japanese B virus ...	4	<i>Bacillus anthracis</i> ...	110
Lumpy Skin Disease virus ...	1	<i>Brucella</i> , all species ...	179
Newcastle Disease virus ...	63	<i>Clostridium botulinum</i> ...	52
Rift Valley Fever virus ...	6	<i>Leptospira</i> , all species ...	41
Rinderpest virus ...	2	<i>Loefflerella mallei</i> ...	17
Saint Louis virus ...	2	<i>Mycobacterium leprae</i> ...	5
Sheep Pox virus ...	5	<i>M. tuberculosis</i> ...	289
Swine Fever virus ...	2	<i>Pseudomonas pseudomallei</i> ...	29
Teschen Disease virus ...	6	<i>Salmonella typhi</i> ...	322
SVD virus ...	1	<i>Vibrio cholerae</i> ...	194
Vesicular Exanthema virus ...	1	<i>Yersinia pestis</i> ...	29
Vesicular Stomatitis virus ...	14	<i>Rickettsia</i> , all species ...	3
Wesselsbron virus ...	2	<i>Coxiella burnetii</i> ...	23
<i>Francisella tularensis</i> ...	3	<i>Chlamydia psittaci</i> ...	28
<i>Histoplasma farciminosum</i> ...	0	<i>Babesia</i> , all species ...	17
<i>Theileria</i> , all species ...	8	<i>Plasmodium</i> , all species ...	16
<i>Trypanosoma cruzi</i> ...	16	<i>M. mycoides</i> var <i>mycoides</i> ...	19
<i>Trypanosoma equiperdum</i> ...	6	<i>M. mycoides</i> subsp. <i>capri</i> ...	16
<i>Trypanosoma vivax</i> ...	12	<i>M. agalactiae</i> subsp. <i>agalactiae</i> ...	17
Category B Pathogens		<i>Coccidioides immitis</i> ...	2
Arboviruses (human) ...	45	Enzootic Bovine Leucosis agent ...	0
Arboviruses (animal) ...	35		
Aujeszky's Disease virus ...	21		

In a report which must surely rate as one of the federal government's most candid assessments of the troubles afflicting nuclear power in the United States, a task force of the Energy Research and Development Administration (ERDA) concluded last week that the nuclear industry faces a stunted future unless public concern over reactor safety, safeguards against the theft of nuclear material and waste disposal are soon cleared up. Moreover, the report also notes that shortages of uranium could restrict the growth of nuclear power after the mid-1990s, that spent fuel is piling up at reactor sites to such an extent that some reactors may soon have to be shut down, and that the government may have to share the financial burden of building new uranium enrichment plants because the nuclear industry is unlikely to raise sufficient capital on its own.

Although those problems are not new, the task force represents a welcome break from many previous government pronouncements on nuclear power, which have mostly followed the line that the problems have been exaggerated by a group of eccentrics who do not know what they are talking about. This report, however, accepts that the "fears of the lay public and its decision-makers are supported by a fair segment of the scientific community—many of whom otherwise support the use of nuclear reactors for the generation of electric power".

On the question of uranium resources, the report notes that unless either plutonium is extracted from spent fuel and recycled as a fuel for light water reactors, or the breeder reactor is brought on line as soon as possible, there is only enough uranium in the United States to guarantee a lifetime's fuel for a limited number of reactors. The task force therefore urges the Nuclear Regulatory Commission (NRC)—which has responsibility for regulating the nuclear industry—to make up its collective mind as soon as possible on whether or not plutonium recycling should be allowed. But, between the writing and publication of the report, the NRC announced that it probably will not make a decision before 1978 on plutonium recycling. The nuclear industry is therefore faced with great uncertainties about how long the fuel for light water reactors will last.

But the NRC's delay in ruling on plutonium recycling has other consequences. Until the decision is finally made, the NRC will not issue a licence for operation of any fuel reprocessing plants, which means that spent reactor fuel will continue to be stored at reactor sites for several more years.

But the task force report notes that 10 reactors do not have any more room for spent fuel storage, and many others will soon face the same problem. The "industry should be advised that additional spent fuel storage capacity is required and that ERDA regards this as an industry responsibility and has no plans for providing such storage", the task force suggests.

On the question of waste disposal, the task force notes that the problems "are tied almost completely to whether or not spent fuel is processed", so that, too, hangs on the plutonium recycling decision. In any case, no method for permanent disposal of radioactive wastes has yet been demonstrated, and the report suggests that problems in

Washington seen

by Colin Norman



finding and demonstrating such a method "together with the lack of decision on the part of the government [have] in fact come close to making waste management the pacing item of the nuclear fuel cycle".

Nevertheless, the task force believes that the problems can be overcome, but that "public understanding and acceptance are essential to the future effective and timely use of fission energy as a source of electric power". The task force therefore calls for "candour and objectivity on the part of the government in dealing with the public". Its own report is a good start.

● Following a number of recent reports in the medical literature that aspirin may help prevent blood clots from forming, the National Heart and Lung Institute has launched a massive clinical study to see whether the drug offers any protection against heart attacks.

During the next year, about 4,200 people aged between 30 and 69 who have previously suffered an acute heart attack will be enrolled in the study, which will be carried out by 30 hospitals and research centres around the United States. Half the volunteers will each receive 1 g of aspirin a day (equivalent to three regular tablets) for three years, while the rest will be given placebos.

The chief objective will be to see whether there is any difference in total mortality between the two groups, and

investigators will also be looking for such factors as differences in the incidence of non-fatal heart attacks and strokes between the two groups. According to Dr William Friedewald, the study director, about 85% of those who now survive one heart attack eventually die from a second heart attack or from other cardiovascular problems.

Volunteers will be given quarterly medical checks while they are taking the drug and more extensive annual checks during a three-year follow-up period. A committee at the Heart and Lung Institute will periodically monitor the study and, unless adverse drug effects show up, the clinical phase is expected to be completed in September 1979.

Aspirin may help guard against heart attacks because it hinders the clumping together of blood platelets, which is believed to be a crucial early event in the formation of arterial blood clots.

● The failure last week of the final stage of a Titan III-C rocket during the launch of a pair of military communications satellites is not expected to affect future space science missions, according to NASA officials. At a meeting held on May 21, the day after the mishap occurred, it was decided that the Viking Mars Lander mission should go ahead in July as planned, even though it will be launched by a rocket whose first three stages are similar to those of the Titan III-C.

Officials of NASA are still not certain what caused the failure, however. Control over the final stage was lost before its engines fired, it began to roll helplessly and eventually burned up on re-entry into the Earth's atmosphere.

The mission, which cost \$57 million, was to have completed a global military communications network consisting of four satellites. The first two were launched in 1973 into stationary orbits over the Atlantic and Pacific, and last week's launch should have placed the other two over the Indian Ocean and the Pacific.

● Virtually unnoted by the general press, or by Congress, two key government positions affecting biomedical research were filled earlier this month. Dr Donald Fredrickson, President of the Institute of Medicine (part of the National Academy of Sciences) was appointed Director of the National Institutes of Health, and Dr Theodore Cooper, a former Director of the National Heart and Lung Institute, was appointed Assistant Secretary for Health—the top health job in the federal government. Both appointments, which were formally confirmed by the Senate, have been warmly endorsed by biomedical scientists.

"ECOLOGICAL considerations are less important than the need to free Israel of dependence on oil producers for her energy supplies," a Government spokesman recently stated in reply to objections from environmentalists to plans for two new power stations, one to be fuelled by coal and the other by enriched uranium. Both represent departures for Israel, where hitherto all power plants have been fuelled by bunker oil. Naturally both pose environmental problems.

First to come into operation will be the plant at Hadera, midway between Tel Aviv and Haifa, which is equipped to burn coal and bunker oil alike. It will eventually have four generators with an output of 1,400 megawatts, roughly equivalent to 80% of Israeli generating capacity today.

Although coal has been discovered in Sinai, its quality is evidently not very high nor is it available in sufficient quantity. More likely sources of supply are Australia, South Africa and the United States. Despite the distances of these countries from local ports, the price of imported coal, presently about \$33 a ton (including transportation), would still be less than oil at \$50 a ton. Officials of the Electric Corporation, which will operate these power stations as it does all others, have promised to tackle coal-related pollution problems by, among other measures, the installation of electrostatic precipitators to remove flyash.

The public is certainly more concerned about the ecological implications of the nuclear power plant than the coal fuelled one, even though, in the perhaps biased words of a physicist involved, "the latter will create pollution problems 100 times more serious than the former."

Public concern has been heightened by the recent statement of nine leading American scientists, five of them Nobel Laureates, urging President Ford to re-evaluate the US Administration's nuclear power programme in the wake of potentially serious accidents at the Decatur, Alabama nuclear power station—accidents that could occur here.

Nevertheless, the 600-megawatt station will almost certainly be built, the most likely site being along the Mediterranean shore some 15 miles south of Tel Aviv. Talks are now going on with firms in the US and Europe about the purchase of a reactor, while the enriched uranium required to fuel it (if, as seems almost certain, it will run on enriched uranium) will presumably come from America in accordance with an agreement signed last year between the government of Israel and the US Atomic Energy Commission. The estimated cost of the installation at to-

day's prices is \$500 million with 60% of the sum, says the Ministry of Commerce and Industry, to be spent in Israel itself on construction, equipment and labour.

● The nuclear engineers required to operate the new installation will be mainly graduates of the Haifa Technion, which has an excellent Department of Nuclear Engineering. Not only has the Department trained new engineers, but it has also retrained engineers already on the staff of the Electric Corporation to handle the problems of nuclear plants.

Ben-Gurion University of the Negev, located not far from the famous Dimona reactor, recently opened its own Department of Nuclear Engineering,

Letter from Israel

from Nechemia Meyers



much to the distress of the Technion, which said it could train all the engineers required at a lower cost. The Haifa school is generally unhappy about the opening of engineering faculties at other institutions of higher learning and about the decision to allow technical colleges to grant academic degrees. Technion Professor Ya'acov Bar has warned that the award of academic degrees to "second-class engineers" would drastically lower the level of engineering in Israel.

● A former Technion lecturer who now holds a demanding industrial position is Dr Dahlia Greidinger, research and development Director at the Chemicals and Phosphates Company, Haifa. Her staff, about half of it female, is concerned with research in two main fields: mineral feed additives to supplement the food of animals and sophisticated fertilisers. In the first category a new product, urea phosphate, is now in semicommercial production, one ton per day being turned out. Also under development is an "instant fertiliser". "In the past few years," Dr Greidinger explains, "work has been done in various countries on liquid fertilisers so

that it would not be necessary to wait for rain or irrigation to bring fertiliser to the plants. But liquid fertilisers are hard to ship or store. So we are developing a solid fertiliser to which the farmer can add his own water when he wants to use it."

● An Israeli chemist recently teamed up with an Israeli historian to throw new light on the construction of Crusader fortifications in the Holy Land. In a report soon to appear in the *Israel Exploration Journal*, Hebrew University historian B. Z. Kedar and Weizmann Institute chemist A. Kaufman give evidence that the mortar used by the Crusaders in their large-scale construction projects contained organic materials, possibly olive oil and flax.

They recall that Rufinus, a little known herbalist of the late 13th century, presented the following recipe for the preparation of mortar: "Grind tiles rigorously and sift them through linsy. Then take smiths' iron-rust and grind and sift similarly, and take tow in the same quantity as of the aforesaid two, and cut it up vigorously with a lopping-knife until it will be triturated on the bench. Having done this, take equal portions of the stone and the iron-rust and put these two and the tow in a mortar, and take olive oil and mix the said cement with the said oil until it is like clay. Apply it at the juncture of the stones and bind the stones together, and let it rest for many days."

They go on to quote an 18th century German missionary Stephan Schultz who, having visited Acre, reported that the extraordinary sturdiness of the Crusader palaces there stemmed from the fact that the builders used oil instead of water in preparing their mortar.

Kedar, the historian, turned to Kaufman, the chemist, to investigate the validity of this story by using carbon-14 measurements to determine whether Crusader-era mortar indeed contained organic materials. Kaufman, a member of a Weizmann Institute team which provides Israel's only carbon-14 dating facility, found that 2% of the material in the mortar from Crusader buildings in seaside Caesarea was indeed of organic origin and dated from AD 1013 ± 160. Whether or not the masons at Caesarea used the Rufinus formula, or even used flax and olive oil is far from clear.

There is no reason to believe that this type of mortar was used only in the Holy Land. French mediaeval literature, for example, contains references to mortar prepared with the blood of serpents or dragons. Unfortunately, present carbon-14 dating methods do not allow researchers to differentiate between olive oil and dragon blood.

The way you lived and died

	Australia	Canada	France	Federal German Republic	Italy	Japan	Netherlands	Sweden	UK	US
Population (millions)	13.1	22.1	52.2	62.0	54.9	108.4	13.4	8.1	56.0	210.4
Crude birth rate (per thousand)	18.9	15.7	16.4	10.3	16.1	19.3	14.5	13.5	13.9	14.9
GDP per capita (\$s)	4,900	5,410	4,900	5,610	2,510	3,760	4,410	6,140	3,100	6,170
Death of infants in 1st year per thousand live births	16.7	16.8	12.9	20.4	25.7	11.7	11.6	9.6	17.5	17.6
Percentage of relevant age group in higher education	28	50	30	16	28	24	20	31	21	44
Grams of animal protein per inhabitant per day	71	66	68	58	45	31	56	58	56	75
Total primary energy requirements in tons of oil equivalent per capita	4.6	8.2	3.5	4.7	2.6	3.2	6.0	4.7	4.3	9.1
Telephones per thousand inhabitants	340	499	199	268	206	315	299	576	314	628

From 'The OECD Observer' March/April 1975.

North to Alaska

from Angela Croome

THE development of large nearshore oil deposits in the North is likely to rationalise the use of shipping in both the American and Soviet Arctic. Since the Americans have so far made no commitment to an Arctic shipping fleet and the projected Alaskan pipeline's cost has now escalated beyond all bounds there is now a good argument for reconsidering freighting under the ice in cargo submarines. Once a fleet of cargo subs had been built the system would appear to be cheaper as well as safer than available alternatives.

The capacity to navigate the polar sea passages across North America and the Soviet Union is not properly used by either nation. The technology for bringing cargo out through the North West passage throughout the year was effectively proved in 1970 with the successful voyage of the supertanker *Manhattan*, but no use has yet been made of this solution. On the other hand, freight is regularly brought out through the Soviet "Northern Sea Route", and plans are in hand to expand the navigation despite the growth of alternative and less arduous land routes.

A North West passage to the Pacific was first attempted as long ago as the reign of Elizabeth I, and the search for it preoccupied many explorers and claimed the lives of not a few. It was in the course of the search for the ill-fated Franklin expedition last century that the route was found though it was not until 1944 that a ship actually got through. The setting up

and supply in the 1950s of the Canadian DEW-line (of defence radars) across northern Canada brought more traffic to the NW sea-route than ever before or since. Fifty ships and 15,000 people were being moved around the Canadian margin of the Arctic Ocean at one time but always escorted by ice-breakers. It had been suggested early on that the NW passage would be more easily negotiated under the ice by submarine. An attempt was made by a First World War US submarine, the *Nautilus*, in 1931 but she got no further than half a hull's length beneath the ice. Nuclear submarines with their much longer submergence times simplified the task and in 1954 the US *Sea Dragon* negotiated the passage entirely beneath the ice. The obvious development now was for cargo-carrying submarines to bring out freight from the Canadian Arctic but there were no bulk products to bring at the time. The situation changed with the proving of oil in Prudhoe Bay, Alaska in 1968, and various alternative transport methods were considered. Ice-strengthened giant tankers which could operate independently of ice-breakers were an attractive possibility and in 1970 an experiment was hastily put together to test the feasibility of this idea. The largest tanker ever built in the United States, the *Manhattan*, jointly commissioned for the job at \$40 million by three oil companies including BP was sliced up and the parts distributed among the shipyards of the North East US for ice-strengthening. (This was partly for speed and partly because no single shipyard could handle such a large ship 307 m long with a 45 m beam.)

Dr Charles Swithinbank now head of the Geology department of the British Antarctic Survey who accompanied the USS *Manhattan* as a member of the glaciological team on its pioneering voyage to Prudhoe Bay in 1970 recently discussed the "modern North West Passage" at a polar symposium at the National Maritime Museum, Greenwich. The object of the experiment was to establish how large a horsepower would be required to drive a 250,000-ton tanker through the ice, and so the *Manhattan* was in fact an over-powered half-scale experiment. The glaciologists made detailed measurements of the ice all along the route which was deliberately chosen to run through the toughest ice—which a normal voyage would avoid. *Manhattan's* passage was triumphantly successful; she had no difficulty in breaking through the thickest ice and regularly preceded her icebreaker escort and a great deal of valuable glaciological and bathymetric data were collected. The conclusion was that it would be feasible for supertankers to service the Prudhoe Bay oilwells 12 months a year.

Nevertheless analyses at the time indicated that bringing the oil out by pipeline would be cheaper, although in the event 800 miles of pipeline have been deteriorating in store at Valdez for several years, and the pipeline cost has escalated to an estimated \$5,000 million. The actual cost of transporting a barrel of oil from Prudhoe by submarine cargo-ship was estimated as marginally cheaper in 1970 at 90 cents a barrel, but of course there was no such fleet available.

An almost opposite situation characterises the North East Passage—the

Soviet Union's "Northern Sea Route" in the account of Dr Terence Armstrong of the Scott Polar Research Institute, who has made a detailed study going back many years. This has been actively operated by the Soviet Union for many years and the route's administration had an almost autonomous existence until the 1930s purges. Some 400 ships ply in the area transporting $2\frac{1}{2}$ million tons of freight annually. Recently a large programme of 24 specially designed medium-sized icebreakers has been laid down in Finland for the route, quite apart from the completion of the hugely powerful new atom-powered Soviet-built icebreaker

Arktika, thought to be capable of 75,000 horsepower. In recent seasons efforts have been made to extend the duration of navigation, limited for the full 'through' route of $2\frac{1}{2}$ months. A complete convoy of 13 ships actually struggled through as late as mid-January in 1972 but a policy speech by the Transport minister subsequently indicated that the experiment proved that the route could be kept open into December but not beyond (giving a September to Christmas season).

The various moves including the large new icebreaker-building programme suggests that the Northern sea route continues to have an important

future despite the impression that it has been losing ground to the "southern" rail route out of Siberia where many extensions and new links have been built in recent years. The freight tonnage of the Northern sea route is holding up well, despite the increased bulk handled by the railway. Dr Armstrong points out that the sea route was developed originally for strategic reasons that never materialised. Nevertheless, having invested heavily in a system in advance of need, he concludes that the Soviet administration is loth to abandon it and expects the Northern Sea route to justify itself until the end of the century. □

correspondence

The reward system

SIR,—A basic fallacy of your Editorial "The Reward System Needs Overhauling" of March 27 1975 is that Fellowships of the Royal Society and Nobel Prizes are not rewards but awards. They are not something a reasonable scientist can ever expect to receive as a matter of course for professional work even of the greatest merit, but a happy chance in which one can rejoice if it should happen to oneself or one's colleagues.

Of course Nobel Prizes "are clearly not enough to go round". Why should they be? They are not a matter of right, a sort of career-grade for the top scientist, but an extra provided by the posthumous generosity of one man. Strictly speaking, their award is the private business of the Nobel Committee and no one else. Admittedly this Committee would be prudent to take account of public standards if confidence in the awards is to be maintained, but your criticism on this score is nullified by your own statement that Nobel Prizes "confer an unreasonable amount of credit on their recipients". Moreover it is invidious to criticise the Nobel Committee for confining their awards "to a restricted number of subjects" when they have broadened their interpretation, within the terms of reference by which they are bound, to include astronomy.

It would have been best if the attack by Sir Fred Hoyle on these astronomical awards had been treated with the contempt of silence. Now that this silence has been broken, it needs to be said that whatever the occasion may have been, these Nobel Prizes are justified at least as much by decades of

distinguished contribution to Radio Astronomy and Cosmology as by any single discovery. Sir Martin Ryle and Professor Hewish had created the physical facilities that made possible the original observations of pulsars, and the background of knowledge that enabled these observations to be interpreted. Mrs Burnell has a secure place in history as the Tombaugh of the discovery, and her credit has been enhanced by the calmness of the remarks she is reported to have made.

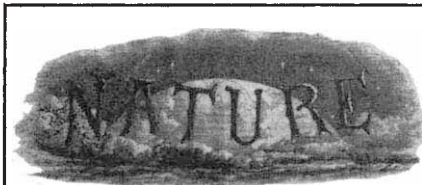
An important lesson from the whole unfortunate matter is that even very distinguished theoreticians may be profoundly ignorant of the realities of discovery in experimental science, and this calls into question the heavy reliance that has been placed on theoretical astronomers in advising SRC and other bodies on strategies for British astronomy.

It may be true that some laboratories make the "pursuit of Nobel Prizes . . . the ultimate aim". If so, the more fools they; in seeking external awards they are missing the true rewards of science. One expects scientists to have something better to do than to display the envy of socialites denied admittance to an exclusive club. If we are indeed seeing "an early shot in a battle which will set scientists against each other in years to come", it will be because they have allowed themselves to be led by journalists whose stock in trade is to whip up "controversy". As one who has negligible chance of ever becoming, let alone deserving, an FRS or a Nobel Laureate, I shall continue to live without envy of those on whom this fortune falls, and I think well enough of my colleagues to believe that the majority of them will

do the same.

P. B. FELLGETT

University of Reading, UK



A hundred years ago

THE REV. G. H. Hopkins gives the following method for fixing the curves which steel filings take when under the action of a bar magnet. The filings having been prepared so as to be as nearly the same size as possible, and that size very minute, are pound into a mortar, and a small quantity of finely powdered resin is added; these are stirred together until the two substances are completely mixed, and then, considerable pressure being exerted upon the pestle, they are rubbed until the resin adheres to the filings in a very fine coating. The filings can then be sprinkled as usual, and the curves formed. It is best (after the curves are formed) to heat the plane surface, glass, paper, or wood, according to convenience, over a stove or in an oven, which easily allow it to be sufficiently as well as uniformly heated. For projecting the curves on a screen, the following, we believe, is a very effective method. Cover the glass with thin gum-water, allow it to dry perfectly; obtain the curves on the dry gummed surface; finally, breathe on the plate: the gum is thereby softened and the curve permanently fixed. Substituting corresponding shaped pieces of paper for the magnets (a pin-hole can be used to indicate the N. pole), the curves can be covered with a second plate of glass, and thus preserved as an ordinary lantern slide.

from *Nature*, 12, 97; 3 June, 1875

news and views

SV40 *A* gene required to maintain transformation?

from a Correspondent

CELLS transformed by certain temperature sensitive (ts) mutants of Rous Sarcoma Virus (RSV) are temperature dependent in their transformed phenotype (Martin, *Nature*, **227**, 1021; 1970; Wyke and Linial, *Virology*, **53**, 152; 1973). Thus RSV viral genes are necessary for the maintenance of the transformed state. The *A* gene of SV40 is known to be required for viral DNA synthesis during the lytic cycle (Tegtmeyer, *J. Virol.*, **10**, 591; 1972; Kimura and Dulbecco, *Virology*, **52**, 529; 1973; Chou *et al.*, *J. Virol.*, **14**, 116; 1974). The *A* gene function is also needed for the initiation of transformation (Tegtmeyer, *ibid.*; Kimura and Dulbecco, *ibid.*) but was thought not to be required for the maintenance of the transformed state (Kimura and Dulbecco, *ibid.*). In the last few months a number of papers have appeared which strongly suggest that cells transformed by ts mutants in the *A* gene are ts for at least some of the phenotypic markers of transformation (Kimura and Itagaki, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 673; 1975; Martin and Chou, *J. Virol.*, **15**, 599; 1975; Tegtmeyer, *J. Virol.*, **15**, 613; 1975; Brugge and Butel, *J. Virol.*, **15**, 619; 1975; Osborn and Weber, *J. Virol.*, **15**, 636; 1975). All these papers confirm that *A* mutants are temperature sensitive for the initiation of transformation.

The cells studied include primary rat (Osborn and Weber), a continuous rat line (Kimura and Itagaki), the continuous mouse cell lines Swiss 3T3 (Tegtmeyer) and BALB 3T3 (Brugge and Butel), Syrian hamster embryo (Tegtmeyer, Brugge and Butel), Syrian hamster tumour cells (Brugge and Butel), Chinese hamster lung cells from a single animal (Martin and Chou), rabbit kidney (Tegtmeyer and Butel), and human cells from a patient with Franconi anaemia (Brugge and Butel). The Chinese hamster lung, rabbit kidney and human cells are semipermissive for SV40 whereas all the other cells are nonpermissive. The *A* mutants used were either isolated by Tegtmeyer (*J. Virol.*, **10**, 591; 1972) (Tegtmeyer, Brugge and Butel; Osborn and Weber), by Kimura and Dulbecco (*Virology*, **52**, 529; 1973) (Kimura and Itagaki) or by Chou and Martin (*J. Virol.*, **13**, 1101; 1974) (Martin and Chou).

In general, cells are transformed at the permissive temperature (usually 33 °C, but 37 °C is used by Brugge and Butel for hamster embryo and human cells) by an *A* mutant and its corresponding wild type. The transformed cells are then tested for their transformed nature at the permissive and nonpermissive (39–41.5 °C) temperatures. The behaviour of the *A* mutant transformed cells are compared to those of wild type transformed (in some cases also cells transformed by late ts mutants) and untransformed cells at these temperatures.

Unfortunately this experimental procedure is not always used. Kimura and Itagaki use cells which have been transformed by wild type virus at 37 °C and which are cultivated at 37 °C until just before being tested. Brugge and Butel use a line of BALB/c embryo cells which were transformed at 37 °C by a different wild type strain (not that from which the *A* mutants were isolated) for comparison with their *A* mutant transformed BALB 3T3 cells. They also use a Syrian hamster tumour line which has also been transformed by a different wild type strain and carried for a long time *in vitro* and *in vivo* to compare with their *A* mutant hamster tumour lines.

Individual transformed clones isolated at the permissive temperature (except for the wild type clones of Kimura and Itagaki) are used by Martin and Chou; Osborn and Weber; Kimura and Itagaki. Both Tegtmeyer and Brugge and Butel used uncloned populations of cells which after infection with virus and subculture at the permissive temperature are deemed to be transformed by their morphology and the presence of T antigen. Thus these two groups are looking at mixed populations of transformed cells.

Criteria for transformation

In general transformed cells are not contract inhibited and grow in a disorganised manner forming dense multilayers and reaching high saturation densities. Normal cells are contact inhibited and grow in an oriented manner usually forming only a monolayer of cells. Transformed cells (especially those not derived from continuous cell lines)

tend to have higher efficiencies of plating (ability to form colonies) than normal cells under a variety of conditions.

The exact definition of a transformed cell has always plagued papovavirus workers and a variety of criteria have been used by the different authors. These include non-contact inhibited morphology (Brugge and Butel, Tegtmeyer, Osborn and Weber), saturation density (Osborn and Weber, Brugge and Butel), colony formation at low cell density in high serum (Tegtmeyer, Brugge and Butel), in low serum (Martin and Chou), in agar (Kimura and Itagaki, Brugge and Butel) and on top of a monolayer of untransformed cells (Brugge and Butel; Martin and Chou), growth rate in low serum (Kimura and Itagaki), presence of T antigen (Osborn and Weber; Tegtmeyer, Brugge and Butel) and increased uptake of hexose (Brugge and Butel).

Using six different *A* mutants Martin and Chou found eight out of nine of their *A* mutant clonal lines are ts for colony formation at low cell density in low serum and for their ability to form morphologically distinct colonies on top of untransformed monolayers. Unfortunately these are the only two tests used and one wonders what happens to colony formation in high serum and if morphological changes do occur at the high temperature.

Kimura and Itagaki find that three independently transformed clones, transformed by one *A* mutant, are ts for colony formation in agar and on top of untransformed cell monolayers. They also report that three clones grow less well in low serum (but not in high serum) at the nonpermissive temperature, but this result is not terribly convincing. Also as stated above they do not use adequate wild-type transformed cells for comparison.

Osborn and Weber present data for only one *A* mutant transformed clone and one wild type transformed clone derived from primary rat kidney cells. They observe that the *A* mutant clone is ts for morphology, alignment of actin fibres, saturation density and the presence of T antigen. Osborn and Weber find that the number of *A* mutant transformed cells containing T antigen

decreases (15% positive) after 3 days at nonpermissive temperature. Tegtmeier and Brugge and Butel do not see any decrease in *A* mutant transformed cells containing T antigen at the nonpermissive temperature, but it is not clear how long they kept their cells at high temperature before testing for T antigen. It is not obvious that Osborn and Weber can fruitfully compare their two clones since the cells they used were derived from heterogenous rat primary cells and different cell types may have been transformed by the different viruses. Many more transformed cells must be isolated and tested before much meaning can be given to these results.

Brugge and Butel study a variety of parameters of their *A* mutant-transformed lines including morphology, saturation density, cloning in agar and on top of untransformed monolayers, the uptake of hexose and the presence of T antigen. They find that all their *A* mutant lines tested are ts for these parameters except for one human line which is not ts for the increased uptake of hexose and that all the lines retain T antigen at the high temperature. Unfortunately not every *A* mutant line is used for every test and as mentioned above some of the wild type transformed cells used are not adequate for comparison with the mutant lines either being of the wrong cell type, being transformed and maintained at the wrong temperature or being trans-

formed with the wrong wild type strain. It is interesting to note that *A* mutant tumour lines which were formed *in vivo* at 37 °C are found to be ts at 39.5 °C.

Tegtmeier's results are somewhat in disagreement with those of Brugge and Butel although the same *A* mutants and in some cases in the same cell types and parameter of transformation are used. He finds that none of his *A* mutant transformed mouse lines are ts for colony formation in high serum. Only rabbit kidney cells transformed by one mutant (A28) are ts for colony formation in high serum (rabbit kidney cells transformed by five other *A* mutants are not ts). The temperature of 41.5 °C, used by Tegtmeier for his rabbit kidney cell experiments, is the highest nonpermissive temperature used by any of these workers. Tegtmeier does find that hamster cell lines transformed by five out of six *A* mutants are ts for colony formation at low cell density, but not at high cell density. All Tegtmeier's *A* mutant transformed lines retain T antigen at the high temperature. He does note that all *A* mutant transformed cells of either mouse, rabbit or hamster that grow at high temperature retain their transformed morphology. Tegtmeier concludes that the temperature sensitivity of the different *A* mutant transformed phenotypes may well depend somewhat on the virus-cell interaction which may vary. Both Tegtmeier and Brugge and

Butel use uncloned mixed populations of transformed cells which contain cells derived from more than one transformation event. It is not clear whether different populations are showing up in the different parameters measured and whether different results would have been obtained if clonal lines had been tested.

Even though there may be some reservations about some of the results presented, and no one paper is entirely convincing, in general all these papers taken together seem to indicate that the *A* gene of SV40 is responsible for at least some of the phenotypic markers of transformation. It certainly seems clear that the induction of transformation by *A* mutants is much more ts than the possible maintenance of the transformed state. Those *A* mutant lines which are not very ts may have been transformed by a wild type revertant, or a less ts variant of the mutant. Alternatively, these lines may possibly contain a spontaneous transformation event (which is not ts) superimposed on the viral transformation or may contain many mutant genomes which together can overcome the ts phenotype. It would be interesting to test the ts phenotype of cells transformed by virus rescued from such lines. It is clear that a lot more careful work will have to be performed before a maintenance function can definitely be assigned to the SV40 *A* gene.

Early history of agriculture

from Don Brothwell

It is something of an event when the Royal Society and British Academy get together to consider topics of mutual interest. The history, more correctly prehistory, of agriculture in relation to developing earlier cultures is such a field of interest. As a veteran symposium attendee, perhaps I should have known that the symposium held at the Royal Society on April 9 and 10 on early man as a farmer would be a disappointment. But some papers were more useful contributions than others; responses were also varied, some being more favourable than my own. The organisers gathered together contributors to cover a wide range of topics, and if the speaker weighting was too heavily towards Cambridge it was at least an example of sample bias which can occur in mammal assemblages related to archaeology.

Following introductory remarks by J. G. D. Clark, J. R. Harlan (University of Illinois) usefully considered the distributions of wild progenitors of domestic animals and plants, as a possible basal reference point from which

to consider the ecological adaptations of domesticated forms. The picture is a complex one, in which it is necessary to appreciate such variables as possible climatic change, the varying influence of human societies, and the extent of further microevolution which might have occurred even in the wild stocks. Harlan viewed patterns of variation among species, attempting a classification into so-called endemic, monocentric, oligocentric and noncentric forms. He emphasised that for a number of domesticates, there had been multiple centres of evolution—a fact as yet not fully appreciated.

W. van Zeist (State University of Groningen), who followed, paid special attention to the early food plants of south-western Asia. The earliest crop plants in this area prior to 6000 BC included barley, wheat, pea and lentil. By 5000 BC the oil plant linseed was also in cultivation. Palaeoethnobotany has its own range of problems, and the nature of ancient plant material presents considerable difficulties in identification of the species. Van Zeist also pointed out

that although pollen studies have been extremely valuable in the reconstruction of ancient environments in this part of Asia, its direct contribution to the study of earlier agriculture is likely to be far more limited.

Some indication of the significance of studying material culture in relation to early domestication and type of economy was given by A. Steensberg (University of Copenhagen). Although an essential part of any reconstruction of early agriculture, he nevertheless pointed out that there were various problems of interpretation—the 'polish' on flint sickles doesn't necessarily indicate cereal cultivation, and so-called paddle-shovels could have had varied uses. Spades and spade marks, he considered, were the first clear proof of husbandry. He reasonably argued that an idea could have remained latent in a community until other conditions were right for its successful application. Thus the antiquity of a positive cultural innovation may not be clearly related to the prehistory of the idea and rationale. Rather surprisingly,

Steensberg was the only one to consider population pressure as a possible determinant of cultural change, and in fact concluded—wrongly I believe—that it has not been a significant factor.

Clearly it was important to have the genetic aspects of crop plant evolution considered in such a symposium, and this was done by Barbara Pickersgill (University of Reading) and C. B. Heiser. They pointed out that domestication led to greater morphological variation than is seen in wild progenitors, but that unique processes were not involved. Moreover, the influence of man as a selection pressure may have changed through time—a fact to be revealed if at all by the archaeological investigation of earlier human communities and their implements. Although cytogenetic aspects of plant evolution have been explored to some extent in relation to wild plants and cultivars, relevant physiological adaptations are by no means worked out. L. T. Evans (CSIRO, Canberra) outlined some of the data and problems in this field—in so far as they might be relevant to studies on ancient cultivars. He indicated that there have been changes in life cycles, degree of environmental control and yield potential in some plants—related to factors of climate, basic anatomy, and hormone variation—but the physiological basis of such changes was not well understood. Human intervention does not seem to have produced an increase in relative growth rate in the various plant species.

In contrast to the previous papers which were mainly concerned with plants, the next two were a slightly limp attempt to restore balance and give more consideration to animal populations.

M. R. Jarman (University of Cambridge) gave general comment on the development of early animal husbandry. He attempted to show the differences between zoo-archaeology and archaeo-zoology, one being concerned with earlier human behaviour in relation to other animal species, while the zoological approach was perhaps more strictly to view the nature of microevolutionary change from wild to domestic forms within a particular species. He emphasised that we must settle into appreciating that animal husbandry in its most general sense occurred well before the Neolithic culture phase. Moreover, it is important to examine the possibility of close man-animal relationships in pre-history beyond the scale of domestic relationships seen today.

As yet biochemical taxonomy has had only modest recognition in helping to resolve questions of domestication. There is no doubt that it has far greater potential, and it was good that at least honourable mention was given to this

Evidence for triggering

from Peter J. Smith

AS long as the nature of deep earthquakes remains unclear, wisdom would seem to dictate emphasis on the study of single-source events. Yet multiple events, though more complex, give additional data which can be put to good use. Both Chandra (*Bull. seismol. Soc. Am.*, **60**, 639; 1970) and Fukao (*Phys. Earth planet. Interiors*, **5**, 61; 1972), for example, were able to use multiple-source earthquakes in the Peru-Brazil region to determine rupture velocities. In each case the secondary events apparently lay on a nodal plane of the primary shock, from which it was assumed that the fault plane was continuous between epicentres and that rupture was planar and unidirectional.

Clearly, however, such analyses beg the question of earthquake mechanism—a problem given point when Dziewonski and Gilbert (*Nature*, **247**, 185; 1974) showed that for some deep shocks the double-couple mechanism is not entirely adequate. Moreover, although Chandra and Fukao obtained relative locations and origin times of

the secondary events, they did not determine the fault plane solutions. This omission could be critical, for Strelitz (*Geophys. Res. Lett.*, **2**, 124; 1975) now shows that it may have led to false assumptions.

Using both P and pP phases, Strelitz has analysed the 5 September 1970 Sea of Okhotsk earthquake which comprised a magnitude 4.5 event followed about 5 s later by a magnitude 5.7 event with roughly the same epicentre but a focal depth 23 km greater. He finds that the fault planes and principal stress axes of the two events were significantly different and that the second event lay on neither of the nodal planes of the first. A curved rupture zone seems to be ruled out on the grounds that such faulting would probably radiate continuously during rupture, whereas the two shocks observed were discrete events. That the proximity of the two events is purely coincidence also seems unlikely because other similar event pairs occur in the same region. Strelitz thus concludes that the first shock simply triggered the second.

field at the conference. E. M. Jope (Queens University of Belfast), in fact, concentrated on animal proteins, and spent much of his allotted time in introducing this complex field. He did however indicate clearly that various lines of investigation may eventually produce significant results, for instance, the haemoglobins and other biochemical variation in modern animal stocks, amino-acid differences in ancient bone, and perhaps even ancient and recent keratins.

Except for the concluding paper, the rest of the contributions were concerned with regions, and in some cases with particular crops. G. H. S. Bushnell (University of Cambridge) outlined the beginnings of agriculture in the Mexican area. Although he is the doyen of Americanists in this country, it was a little regrettable that those who had more direct acquaintance with the significant developments in the agricultural history of the New World were not in attendance at the meeting. Bushnell did make the pertinent point that the long, and probably ecologically complex, development of agriculture in the Americas could hardly be seen as a "revolution" in Childe's sense of the term.

Sir Joseph Hutchinson's task was to consider crop plant evolution in the Indian sub-continent. He indicated that the various plants of economic value to the earlier cultures had a diverse origin

—being introduced at various times from Asia to the east and from both south-west Asia and Africa to the west. Tea was one of the few crop plants which was probably indigenous. Possible trans-Pacific movement of plants was considered in relation to maize. In contrast to these plant origins, Hutchinson believes that livestock may not have been so derived from elsewhere—wild forms of domestic varieties being still seen in India. Early agriculture in this area, Hutchinson suggests, is best seen as an extension of that evolved in Western Asia, and certainly there were links into Mesopotamia. Finally, Hutchinson pointed to the considerable variation which had occurred in some plants, such as the banana, cotton, wheats and rice—not so much an indication of the degree of domestication as the nature of the breeding system, length of time the crop was under cultivation, the cumulative nature of certain mutations, and even the response to disease. Further aspects of early Asian agriculture, and particularly rice cultivation, were explored by T. T. Chang (International Rice Research Institute, Philippines). As he said, rice now feeds more people than any other cereal and clearly has an intimate relationship with the development of some cultures, especially in eastern Asia. Varieties of rice appear at different dates. In some rice cultures, millet was in fact the oldest crop; an

alternative being mixed cropping as an insurance against climate failure. Intimately bound up with the success were the emerging techniques of irrigation, terracing, fallowing, weed control and manuring. A major question in resolving the development of rice cultivation is concerned with the contrasting styles of upland and lowland rice cultures, although their relative antiquities, Chang feels, cannot be resolved at present.

The contributions given by E. S. Higgs and Heather Jarman (University of Cambridge) were both concerned with the development of prehistoric agriculture in Europe, either in the uplands or lowlands (where resources tend to differ from one another). Both were involved with the construction of a theoretical framework concerned with possible changes in relations between populations, natural resources and the level of technology. Patterns of agricultural exploitation, they consider, can be demonstrated from divergent regions of Europe. This territory approach is one of the methods particularly adopted by the Cambridge group, and deserves fully testing on actual site data. Unfortunately theoretical frameworks, although worth exploring, are always in danger of showing the 'average' rather than the highly variable nature of human society.

As a classical archaeologist, J. Boardman (University of Oxford) provided something of a contrast to the majority of other papers. He demonstrated that this zone of archaeology can contribute usefully to a full reconstruction of plant use in relation to earlier human cultures. Although the olive was dealt with in particular detail, various plants and animals could have been similarly treated. Wild olive grew freely in the early Mediterranean, and its value was well recognised by Bronze Age times—when it became important economically in Greece at least. The elaboration of a material culture to cope with the extraction and uses of olives even included special scrapers for the removal of oil after its application to the body!

The concluding paper of the conference provided a worthwhile contrast, more precisely an alternative viewing of the relationship between man and his environment during these earlier cultural phases. G. W. Dimbleby (University of London) pointed out that although during his evolution man had 'freed' himself to some extent from his natural environment, he has probably influenced it profoundly in some areas—although to a varying degree depending on the nature of the human activity within the ecosystem. In particular agriculture, both pastoral and arable, has been a significant imposed change on the ecosystem—with resultant changes to the components of the

microclimate and vegetation. Eventually, even soil degradation and marked erosion can occur, and then it is increasingly difficult for regeneration processes to occur. Holocene environments, then, are by no means in a state of equilibrium, and some are far more 'brittle' than others. It would also appear that often the early farmers were in fact concentrating on these brittle environments, such as savanna woodland, steppe, or light deciduous forest—and there is growing evidence of the detrimental side effects which eventually occurred.

X-ray diffraction studies of biomembranes

from A. E. Blaurock and W. R. Lieb

MEMBRANE biochemists, following the maxim that "the proper study of mankind is man", have studied the human red blood cell membrane in great detail. Unlike other human tissues, blood can be obtained in large quantities without harming the donor. There is the added attraction that the red cells have no internal membranes to complicate matters. And pure cell membranes are easily obtained by haemolysis, that is by osmotically bursting the cells and extruding their contents into hypotonic solutions. The resulting 'ghosts' are

single-walled sacs of cell membranes.

It has been found that most of the ghost protein will react with aqueous labelling reagents and enzymes when these are allowed to enter the sac, but little reacts when these are kept outside. The inference which membrane biochemists have drawn from such results is that most of the protein is associated with the inner, cytoplasmic surface of the membrane (for a recent review, see Steck, *J. Cell Biol.*, **62**, 1-19; 1974). In addition, about a third of the total protein mass is released at very low ionic strength without detergents, suggesting a location largely extrinsic to the lipid bilayer. Interestingly, this superficial protein (mainly 'spectrin') reacts exclusively with inside reagents. The picture that emerges is of a very asymmetric membrane, with more protein on the inside than on the outside and with much of this inner protein lying superficially at the cytoplasmic surface. With this picture in mind, it is surprising that a recent X-ray diffraction study by Stamatoff, Krimm and Harvie (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 531-534; 1975) concludes that the electron density profile for the ghost membrane is symmetrical.

Although ghost sacs in unoriented dispersions have been looked at by X-ray diffraction, Wilkins *et al.* (*Nature new Biol.*, **230**, 72-76; 1971) also pointed out the advantages that would come from arranging the membranes in

A new anti-sickling agent?

from a Correspondent

CURRENT interest in the undamental aspects of the aggregation of deoxy sickle-cell haemoglobin is but one approach to the serious practical problems which arise in the management of sickle cell anaemia patients. Side by side with studies on the behaviour of deoxy Hb-S there have been attempts to devise therapeutic methods for reducing or abolishing the sickling process *in vivo*. Proposals to use urea and, more recently, cyanate as anti-sickling agents have been widely publicised and their effects on the aggregation of deoxy Hb-S are susceptible to rational explanation. The value of cyanate as a long-term anti-sickling agent is now being very carefully assessed by US workers. Less has been heard recently of the anti-sickling properties of steroids, although both testosterone and progesterone have been tested *in vitro* and *in vivo* and claimed to have some degree of efficiency.

Now comes the news of an entirely novel type of anti-sickling agent, found in aqueous extracts of *Fagara zanthoxyloides* root. In their

first publication in a European journal, a group of workers in Ile-Ife and Ibadan, Nigeria, report some toxicological and preliminary clinical trials of this material (Isaacs-Sodeye, Sofowara, Williams, Marquis, Adenkunle and Anderson, *Acta haemat.*, **53**, 158; 1975). The roots in question are used as chewing sticks in the Western State of Nigeria and then the fibrous end thus produced is used to brush the teeth. There is evidence of antimicrobial activity against oral microorganisms in the root extract and no folk history of side effects.

The paper intimates favourable toxicological findings in limited tests on *F. zanthoxyloides* extracts, and a significant diminution in the incidence of painful episodes (sickle-cell crises) in the few patients treated. Comprehensive clinical trials at many centres in Nigeria are now being organised. The results of these trials will be awaited with great interest, especially as it is stated that a pure component of the root extract with potent anti-sickling activity can also be synthesised in the laboratory.

regular, oriented stacks. Even if the membrane is asymmetric, the collapsed sac will then form a symmetrical structure, greatly simplifying the determination of the membrane electron density profile. In addition, in-plane diffraction is then seen separately from profile diffraction. These considerations were also in the minds of Stamatoff *et al.*

Their ghost membrane sacs were prepared in the usual manner. To orient the membranes, the sacs were then spun for a long time at a very high speed (24 h at 100,000 g, 4 °C), and excess water was removed by equilibrating in a nitrogen atmosphere at high relative humidities. The spacing between membranes was varied by a final equilibration at 75–100% relative humidity. The presence of sharp diffraction arcs showed that these preparations indeed contained oriented membranes in regular stacks. The repeat distance was 56 Å at the lowest hydration, increasing to 70 Å when more water was present. If, as the biochemists argue, the membrane profile is asymmetric, one expects the repeat distance to include two membranes, since the collapsed sac is then the symmetrical unit. But current ideas of biological membranes are based upon a bilayer arrangement of the lipids, and a bilayer thickness of less than 28 Å is unlikely. Therefore the authors reasonably conclude that there is only one membrane in the repeat unit, with a thickness of some 56 Å. Assuming that the ghost sacs are intact, they further conclude that the erythrocyte membrane has a symmetrical electron density profile.

At present, then, there seems to be a contradiction between this interpretation and the biochemical findings, and one must look for ways of reconciling the experimental results. The authors point out that, in general, a membrane which is biochemically asymmetric may nonetheless have a symmetrical electron density profile. But the weight of biochemical evidence points to there being much more protein on the cytoplasmic than on the extracellular side of the ghost membrane. It may be that the biochemical evidence has been misinterpreted. But it is more likely, in our view, that the native structure of the ghost sacs was altered during the fairly vigorous steps required to orient the membranes. Knutton *et al.* (*J. Cell Sci.*, **7**, 357–371; 1970), in a similar X-ray study, did report such alterations, although the procedures were not identical to those of Stamatoff *et al.* Support for this possibility also comes from the latter group's calculated electron density profiles. They are rather similar to those published for egg lecithin/cholesterol bilayers (Levine and Wilkins, *Nature new Biol.*, **230**, 69–72; 1971). The peaks at the presumed lipid headgroup regions are narrow. In addition,

when much of the water is removed from between the membranes, peaks in neighbouring profiles come right up to each other. Thus there is little indication of the large amount of superficial protein, such as the 'spectrin', present in the native membrane.

Clearly, for the future, it would be very valuable to study possible changes in membrane structure during each stage of the preparation of oriented specimens. For example, electron microscopy could be used to check for fragmentation of the ghost sacs. Since most biological membranes are not naturally oriented, a successful resolution of these difficulties would greatly extend the application of X-ray diffraction techniques.

How do animal cells communicate?

from John D. Pitts

An International Titisee Conference on "Information Transfer between Cells", sponsored by the company of Karl Thomae and chaired by W. R. Loewenstein (University of Miami), was held on April 24–27.

DIRECT communication between animal cells through junctional channels which join the cytoplasms of adjacent cells has now become accepted as one of the important and fundamental mechanisms of intercellular communication. Communication is necessary for the coordination and control of growth, pattern and function in multicellular systems and the different forms of communication, including surface-surface interactions, communication by extracellular signalling molecules and junctional communication, may all play a part.

Small molecules and ions move freely through intercellular junctions which means that the cell has lost its status as the independent unit of animal tissues, though its individuality is maintained by its macromolecules which do not pass through junctions and are therefore restricted to the cell in which they are made. At this interesting time, when ideas and attitudes are being modified, the Titisee conference sorted out some of the ground rules and established from anatomical, physiological and biochemical approaches what are the generally accepted properties of junctional communication.

Junctional permeability can be detected either electrophysiologically or biochemically and the site of permeability has been correlated with the morphologically identifiable gap junction. These junctions appear as arrays of closely packed (hexagonally packed

in fixed preparation) subunits, 80–90 Å in diameter which traverse the two closely apposed membranes. N. B. Gilula (Rockefeller University, New York) and D. A. Goodenough (Harvard Medical School) described the structure of the junctional subunit as a dumb-bell-shaped protein complex penetrated along its length by an aqueous channel 15 to 20 Å in diameter. The junctional proteins are not glycosylated and can be separated into two components of molecular weight 25,000 and 15,000.

The permeability characteristics of these junctions are consistent with the idea of non-specific transfer through simple aqueous channels. Electrophysiological measurements show free movement of ions between cells joined by gap junctions (hence the term low-resistance junctions). Injected dyes such as fluorescein diffuse through junctions and J. D. Pitts (University of Glasgow) reported that nucleotides, sugar phosphates, choline phosphate and the co-factor tetrahydrofolate are exchanged freely between junction-forming cells in culture. So the picture emerges that many (perhaps all) small cellular ions and molecules pass through junctions. But there is a size limit on transfer: junctions are not permeable to proteins or nucleic acids, although in contradiction to Pitts, G. M. Kolodny (Harvard Medical School) claimed that RNA is transferred between mouse 3T3 cells, but only in very confluent culture.

Evidence for modulation of junctional permeability was presented by B. Rose (University of Miami) and W. R. Loewenstein. They have correlated a reversible increase in junctional electrical resistance with transient increases in intracellular calcium concentration. The idea of controlled permeability is attractive although as yet there is no evidence for such control occurring under physiological conditions.

Intercellular growth control mediated by junctional exchange of metabolites has been observed in simple culture systems but data by M. G. P. Stoker (Imperial Cancer Research Fund, London) suggest that not all forms of growth control depend on junction formation. The loss of topoinhibition at wound edges in culture is apparently not due to loss of junctional communication, so it seems, perhaps not surprisingly, that more than one form of communication is involved in cell growth control.

Another important form of direct communication between cells is mediated by surface-surface interactions which may well involve carbohydrate groupings. Y. C. Lee (Johns Hopkins University, Baltimore) and H. Wiegant (Philipps University, Marburg) reviewed the structure and properties of

membrane glycoproteins and glycolipids and R. C. Hughes (NIMR, Mill Hill, London) described cell variants with specific glycosylation defects. Such cells will provide valuable tools for studying the functions of cell surface carbohydrates. M. M. Burger (University of Basel) described a proteoglycan-type aggregation factor and a second factor (termed baseplate) isolated from sponge cells. If baseplate is covalently linked to sepharose beads, the beads aggregate in the presence of aggregation factor. Further analysis of this simple system could lead to an understanding of the molecular mechanisms involved.

The role of cell movement and cell contact in embryogenesis was emphasised by A. Moscona (University of Chicago) who suggested that re-aggregation of dissociated cells provides a useful model system for the study of this process. He has isolated tissue-specific glycoprotein ligands (cognins) which mediate specific cell reaggregation in the chick neural retina system.

Communication mediated by extracellular factors may also play an important part in growth control and differentiation. W. J. Rutter (University of California, San Francisco) described the effect of a factor isolated from mesenchyme which can regulate the differentiation of pleiotropic precursor cells in primitive rat pancreas. The factor seems to act at the cell membrane and has two functional components. One, which is periodate sensitive, acts by increasing internal cyclic AMP and the other has a separate but unknown function. M. C. Raff (University College, London) pointed out that there are two classes of extracellular signalling ligand, those which act monovalently altering the activity of a membrane receptor and those which act multivalently causing receptor clustering. In two cases of signal transduction by receptor clustering—mast cells treated with specific antigen or anti-IgE and T lymphocytes treated with certain mitogens—there is a rapid (in less than a minute) and transient increase in calcium uptake (see page 378 of this issue). Raff suggests that ligand binding opens membrane calcium channels and that increased cellular calcium levels initiate (in some unknown way) the further events leading to histamine release or DNA synthesis.

In the slime mould G. Gerisch (Friedrich-Miescher Laboratory, Tübingen) has shown that extracellular cyclic AMP pulses stimulate the cellular cyclic AMP generating system by a feedback mechanism and thus the necessary amplification is provided to relay cyclic AMP-mediated signal from one cell to the next. This type of mechanism for maintaining signal level along a chain of cells could clearly be

of more general importance.

The specificity of synapse formation must depend on some form of intercellular communication, as yet unknown. A number of people have approached this central problem of neurobiology from different angles. J. G. Nicholls (University of Stanford) has defined the detailed cellular organisation of leech ganglia and is studying synapse regeneration in organ culture. M. Nirenberg (National Institutes of Health) has isolated and partly characterised a number of neuroblastoma cell lines with defective neural gene products. J. P. Changeux (Institut Pasteur, Paris) has made a detailed study of the cholinergic receptor protein. C. Levinthal (Columbia University) has described, in three dimensions, the growth of particular nerve fibres towards specific target neurones or neuroblasts. M. Jacobson (University of Miami) has studied the temporal sequence of events leading to the establishment of specific retinal tectal connections in amphibia. S. Ward (Caltech) has isolated behavioural mutants of a nematode which fail to respond to a variety of chemical attractants. Anatomical changes have been identified in the terminals of the sensory neurones of mutant worms in four out of six complementation groups. Mutants in three of these groups are sterile so if sterility and the sensory change result from the same molecular defect, then the biochemical analysis of sperm (which are easily isolated) may lead to an understanding of the neurological defect.

The concept of successive subcompartmentation during insect development was reviewed by P. Lawrence (MRC, Cambridge). The division of one compartment into two subcompartments appears to be associated with the expression of a single gene in one subcompartment. The *engrailed* gene, expressed by cells in the posterior wing compartment of *Drosophila* distinguishes these cells from those in the anterior compartment. Clones of rapidly growing cells with a defective *engrailed* gene (produced by genetic tricks) which appear in the anterior compartment respect the anterior-posterior compartment boundary, whereas clones appearing in the posterior compartment do not.

The formation of gradients of positional information, which specify pattern during development, also depends on intercellular communication. If it depended on the movement of signal molecules through intercellular junctions, it might perhaps be expected that restricted permeability should be found at the compartment boundaries. Lawrence has examined the insect segmental boundary and finds no electrophysiological or morphological evidence

for discontinuity of junctional coupling.

A. Gierer (Tübingen) proposed that gradients of chemical factors, rather than intrinsic cell polarity, determine the polarity of regeneration in *Hydra*. L. Wolpert (Middlesex Hospital Medical School, London) suggested that in the developing chick limb position along the antero-posterior axis is probably specified by a gradient of information originating from a region near the posterior margin, but that along the proximo-distal axis position is specified by the time cells spend (number of divisions made) in a progress zone at the growing tip of the limb bud.

Intercellular communication is clearly important in multicellular organisation, but which form of communication has which role is yet to be determined.

Interfering with cancer

from Mathilde Krim, Arthur S. Levine, Thomas C. Merigan and Jan Vilcek

An international workshop on interferon in the treatment of cancer, held in New York on March 31–April 2, was sponsored by the Memorial Sloan-Kettering Cancer Center and the Division of Cancer Treatment of the National Cancer Institute. The meeting was attended by workers from a number of European countries and by many American workers, including representatives from the Institute of Allergy and Infectious Diseases, whose anti-viral programme has sustained work in the USA for several years.

INTERFERON, a naturally produced anti-viral protein, inhibits the replicative and transforming activity of both DNA- and RNA-containing tumour viruses. But its *in vivo* effect on tumours apparently cannot be explained solely by its anti-viral action. Ion Gresser (Institut de Recherches Scientifiques sur le Cancer, Villejuif) and others reported that mouse interferon delays the appearance or retards the spread of mouse virus-induced leukaemias and certain solid tumours, in some of which there is no demonstrable viral involvement.

One investigator reporting at this meeting, however, Richard Adamson of the National Cancer Institute, stated that he had been unable to demonstrate an anti-tumour effect in his L1210 mouse leukaemia system. But interferon has been shown to be capable of protecting mice in instances of tumour induction by radiation and by the chemical carcinogen methylcholanthrene. Should interferon have anti-tumour

effects *in vivo* operating through mechanisms other than those concerned with inhibition of viral proliferation, experimental evidence has suggested that these could be: inhibition of tumour cell division, changes in tumour cell surface properties, modulation of lymphocyte functions, enhancement of macrophage phagocytic activity and changes in serum complement level. The relative importance of these effects is still unknown; in some cases interferon might act directly on the tumour cells; in others it seems to modify components of the immune system.

Martin Hirsch of the Massachusetts General Hospital in Boston reported that in chimeric mice (models for human organ and bone marrow transplantation) mouse interferon reduces C-type virus production, lymphoma incidence and the severity of graft-versus-host reactivity. He and Edward De Maeyer, Institut du Radium, Orsay, have also shown that it delays skin graft rejection.

In the 1960s, a number of clinical trials with synthetic interferon inducers administered to patients were inconclusive in that inducers were found to have both toxicity and limited effectiveness with regard to the level of interferon they elicited in man. The alternative, the large-scale production of exogenous human interferon by cultured cells, was pursued in a number

of laboratories, particularly at the Central Public Health Laboratory in Helsinki. Here Kari Cantell has used leukocytes obtained from blood bank buffy coats as interferon producers, and has perfected a system of obtaining high titre interferon preparations suitable for clinical use. Now, with Hans Strander of the Karolinska Institute, he has begun to explore the use of his product in the treatment of a variety of human cancers.

Late in 1974 Strander reported that in a small group of patients with osteogenic sarcoma treated over the past two years surgical removal of the primary tumour, followed by long-term administration of interferon, seemed to have warded off the occurrence of metastases (National Cancer Institute Monograph, in the press). The high titre exogenous interferon he uses seems to be devoid of overt toxicity.

These early results provided the impetus for the workshop, which was designed to review them critically. A careful review by Dr Strander of his clinical evidence was followed by the report by Alan Rabson (National Cancer Institute) on two independent reviews of the pathology of the treated Scandinavian cases. The originally malignant nature of all the tumours was confirmed, but it was felt that statistical analysis could be based only on that bone tumour type for which solid prog-

nostic data exist, agreed by all to be classical osteogenic sarcoma. It was concluded that the rate of recurrence of disease in the form of lung metastases in the cases so selected did appear reduced following interferon administration (with bordering statistical significance because of the small sample size and the relatively short follow up) when compared to the rate of recurrence among Swedish historical controls or, for that matter, the generally observed rate of recurrence.

Emil Frei, Director of the Sidney Farber Cancer Center in Boston, then observed that it is not yet clear how the Scandinavian results will compare to those obtainable with recent effective chemotherapy, but that interferon's lack of toxicity could be considered a distinct advantage if it were to have clinical usefulness. It was agreed that the clinical trials in osteogenic sarcoma should be expanded.

The implication of this is that more human interferon must be produced.

A session of the workshop concerned with new methods of interferon production indicated that this expansion of production can be achieved by the application of modern technologies in large scale cell culture. The interferon so produced would still be insufficient for wide use. Thus biochemists must pursue human interferon's purification, sequencing and, ultimately, synthesis,

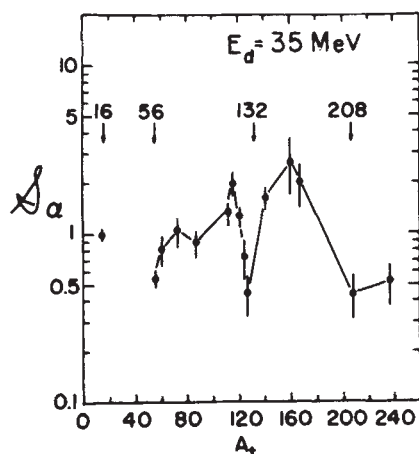
THERE is much current interest in alpha-transfer reactions (*Nature*, **252**, 270; 1974) because of the information they can give us about alpha-particle clustering in the nuclear surface. There are many reactions that either remove an alpha particle from the nucleus or give an alpha particle to the nucleus, and among these the (d, ^6Li) reaction is particularly convenient because it is relatively easy to produce intense deuteron beams and to detect the emitted ^6Li ions.

This reaction has recently been used by the Michigan group (Becchetti *et al.*, *Phys. Rev. Lett.*, **34**, 225; 1975) to make a systematic study of alpha pickup in a range of nuclei from ^{12}C to ^{238}U . They irradiated these nuclei with 35 MeV deuterons, and observed the ^6Li emitted at the angle corresponding to the maximum in the cross section for $L=0$ transfer. They found that the cross section falls rather steeply as the target mass increases (roughly as A^{-3}), with superposed fluctuations that are connected with the nuclear shell structure.

To study these variations in more detail, they calculated the cross section for alpha-particle pickup using the distorted wave theory. This contains a factor S_α that is a measure of

Alpha-transfer reactions

from P. E. Hodgson



Alpha-particle spectroscopic factor S_α obtained from analyses of (d, ^6Li) reactions normalised to unity at ^{16}O and plotted as a function of target mass.

the probability of finding an alpha particle in the target nucleus. The resulting values of S_α , normalised to unity for ^{16}O , are shown as a function of A in the figure.

The fluctuations of S_α with A can be correlated with the nuclear shell

structure in a very simple way. The minima correspond to nuclei with closed neutron or proton shells, and the maxima correspond to nuclei with open shells. Thus the maximum around $A=110$ corresponds to open neutron shells and that around 160 to open neutron and proton shells.

These results are closely parallel to those found by Milazzo-Colli and Braga-Marczhan in their studies of (p, α) and (n, α) reactions (*Nature*, **245**, 12; 1973), and of alpha decay. The probability of alpha emission is given by the product of the probability of finding an alpha particle on the nuclear surface and the probability that it can penetrate through the potential barrier of the emitting nucleus. Analysis of data on many spontaneous alpha decays gave the variation of the alpha preformation probability with A (Bonetti and Milazzo-Colli, *Phys. Lett.*, **49B**, 17; 1974). Their result was similar to that shown in the figure and in particular showed a marked minimum in the region of the doubly closed shell nucleus ^{208}Pb .

Taken together, these results provide useful additional evidence concerning the probability of finding alpha particles on the nuclear surface.

which would be also greatly facilitated if more human interferon were available.

The workshop ended with a consensus that a broad-based international collaborative programme of laboratory and clinical work should be intensified, aimed first at establishing whether interferon has anti-tumour potential in osteogenic sarcoma, and then at exploiting its whole range of clinical potential.

Neglected research and the power of bureaucracy

from Kenneth Mellanby

The Council for Science and Society held an open forum on "Neglected Research and Social Priorities" in London on May 3, in order to identify neglected fields of research which might be of value to man and to society.

MAGNUS PYKE described the various actions the government had taken during and after the last war, with the intention of improving the health of Britain's population. He pointed out that the government was still insisting on considerable expenditure to fortify bread with calcium and vitamins, though no research had been done to find out whether these additives were actually doing good. We do not even know to what extent the improvement in health during the war was due to higher wages or to governmental benevolence.

Energy was the concern of Walt Patterson of Friends of the Earth. He believed that the government lacked a proper energy policy, and that more work should be done on such topics as wind, solar energy, bioconversion, advanced coal extraction technology and less on nuclear problems. The next speaker, Colin Tudge, was also primarily political. His topic was "What value is agricultural research?", and his conclusion was, "very little". His main concern was that most research supports western capitalism, and that the implementation of a Marxist system could produce better results and make most of this research unnecessary. He alleged that research on tropical crops of value to the third world was starved. This conclusion was not borne out by a report I received through the post on the same day, listing the lavish expenditure of the International Institute of Tropical Agriculture at Ibadan, Nigeria, where ambitious projects on yams, sweet potatoes, cassava, maize and tropical soil conservation are in progress.

John Williamson of Guy's Hospital

wanted more research into medical efficiency. He deplored the lack of effort given to common complaints like acne, cystitis and asthma, and on comparisons between self-treatment and that given by skilled clinicians. Nigel Calder, who spoke as a concerned journalist, was worried that the damage done by Lysenko to Russian genetics might be paralleled by the scope for obtuse though powerful scientists to block the supply of both public and private funds to fields of which "the establishment" disapproved. He cited the case of Professor Lamb's Climatology Unit in the University of East Anglia, which, even after prolonged negotiations failed to obtain support from any of the research councils; fortunately, in the event, it has been temporarily saved by private foundations. Calder compared unfavourably the many millions spent on meteorology, where the largest and most expensive computers have possibly made marginal improvements in the two and three day weather forecasts while, in Britain, climatology is almost totally neglected.

Eve Goss described, from her personal experience, the difficulty of the disabled in obtaining help from the authorities, and suggested that more research on how the old and disabled may be integrated into the community was desirable.

The open discussion was introduced by Colin Fisher of the Pye Charitable Trust, who listed a series of neglected areas, and tried to explain the reasons for this neglect. These included laziness and elitism in research workers themselves, and conservatism in the technocrats. He queried the wisdom of allowing the financing agencies to have so much power in stimulating research in their chosen fields. He showed how some of the private foundations may rely on the same advisors as the governmental organisations, so that the same dogmas are perpetuated. He made a plea for a more agnostic approach, and for at least some support for whimsical problems.

H. H. Lamb explained the problems, largely financial, in starting new work in Britain. He gave chapter and verse for instances of totalitarianism in laboratories where workers were compelled to study projects they knew would prove unproductive, and were forbidden to follow lines which, later, proved to be important. He thought that the provision of some funds from private sources, from industry and from American and international sources was some insurance against the tyranny of the establishment.

Various speakers then described specific topics; there was some interest in Tony Benn's suggestions (made when he was a member of the govern-

Contact between photoreceptors

from a Correspondent

THE idea that each vertebrate photoreceptor acts independently of other cells has long been explicit in accounts of visual function. Yet for over a decade there has been anatomical evidence for connections between receptor cells (Sjostrand, *J. ultrastruct. Res.*, **2**, 122-170; 1958). In the last ten years, intracellular recording methods have advanced to the point where a full study has been made of turtle receptors; in particular, the electrical responses of cones have been modelled and a description has been made of local effects transmitted from other cones, and of a distant antagonistic effect mediated by horizontal cells. It seems that colour and pattern processing, and adaptation phenomena, begin at the most peripheral level in the visual pathway (Baylor, Fuortes, and O'Bryan, *J. Physiol., Lond.*, **214**, 265-294; 1971; Baylor, Hodgkin, and Lamb, *J. Physiol., Lond.*, **242**, 759-791; 1974).

Physiological evidence has also been presented that rod receptors are extensively coupled (Fain, *Science*, **187**, 838-841; 1975; Schwartz, *J. Physiol., Lond.*, **246**, 617-638; 1975). In addition, J. E. Dowling has described evidence (at the April meeting of the British Photobiological Society) of extensive 'fin-contacts' between rod receptors at the level of the outer limiting membrane. It seems that functional coupling between neighbouring rods may be used to signal single quantal absorptions in the face of the various sources of receptor and transmission noise. Though the functional significance of the different varieties of inter-receptor contacts remains to be studied, it is clear that they have an important effect, and that our ideas of receptor excitation have undergone a brisk change.

ment in 1966) that some research funds should be available to trade unions and consumer groups.

The forum did not reveal any vast fields where profitable work was needed, and where neglect was total. It did reveal concern at the growing power of bureaucracy, and a danger that the original but unorthodox worker was finding it increasingly difficult to get support. To me the most worrying cases were where, in some universities and research institutes, workers were actually forbidden to tackle problems even when no extra funds were needed.

articles

Salt floors to geosynclines

David J. J. Kinsman

Department of Geological and Geophysical Sciences, Princeton University, Princeton, New Jersey 08540

I propose that if young rift oceans (proto-oceans) are commonly the site of large scale evaporite deposition and if the sedimentary prisms of rifted margins are correctly identified as eugeosynclines, then it follows that geosynclines should commonly be floored by evaporites.

UNTIL a decade ago the linear belts of thick sedimentary rocks, commonly referred to as eugeosynclinal deposits, were generally thought to have accumulated within the craton on subsided sialic, or continental type crust. Hess¹ and Dietz^{2,3} suggested that eugeosynclines were initially ensimatic and only later in their history were welded on to and into the continental craton. In the context of seafloor spreading Dietz proposed that the prism of sediments which accumulated along a rifted or trailing continental margin represented the early phases of eugeosynclinal accumulation. Deposition occurred partly on the thinned and submerged edge of the continent (the continental slope) but mostly on the adjacent oceanic crust to form the continental rise. Thus a relatively long, tectonically quiet stage of sediment accumulation, representing the trailing history of a continental margin, precedes the more tectonically active stage when the margin uncouples and oceanic crust is subducted. Most trailing continental margins can be envisaged as having undergone a history of: (1) initial intra-continental plate rupture, with formation of a new plate boundary; (2) continued seafloor spreading with addition of oceanic crust adjacent to the continental margin, moving the margin to an intraplate position; and (3) later uncoupling and new plate boundary inception between continental and oceanic crust at the continental margin. The maximum ages of trailing continental margins and of ocean floors suggest that the tectonically quiet stage of sediment accumulation may be as long as 150–200 Myr, but this value probably depends on long term, average, global spreading rates.

Sclater and Francheteau⁴ have demonstrated that most new oceanic crust generated at spreading ridges starts life between 2 and 3 km below sea level and subsequently follows a time dependent subsidence, related to heat loss and thermal contraction. An average depth-age curve for oceanic crust is shown in Fig. 1. This curve describes the depth history of sea floor when loaded with water and a negligibly thin veneer of sediment. Hot spot or plume generated⁵ sea floor may, however, be formed at shallower depths or even subaerially, although it also apparently follows a similar time dependent subsidence^{6,7}. Sleep⁸ has used a similar thermal model to explain the subsidence rates of North Atlantic rifted continental margins and Kinsman⁶ has also used this thermal model to describe the uplift, rifting and subsidence history of rifted continental margins. Assuming isostasy at all times and combining sediment density-depth data with the thermal subsidence model, it is possible to determine the maximum marine sediment loading which can be accommodated on rifted continental margin of various crustal thicknesses and on ocean floor, both with

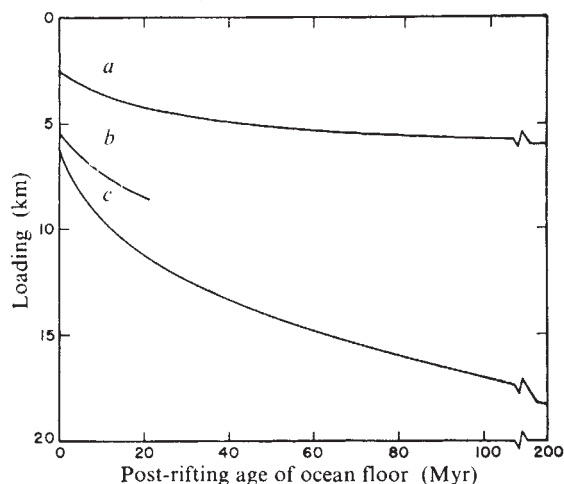
reference to their post-rifting age⁹. The maximum loading by marine sediments increases seaward off the continental margin as continental crustal thicknesses decrease and is greatest on oceanic crust of normal thickness. The maximum marine sediment loading of oceanic crust increases with post-rifting age of the crust but is predicted using this loading model to be close to 16–18 km for the range of average sediment densities (Fig. 1). Along trailing continental margins thicknesses of this order are found under several of the world's largest deltas where sedimentation has now definitely stepped well clear of the continental margin and oceanic crust is being deeply buried (examples include Niger delta⁹—14 km; Mississippi delta¹⁰—16–17 km; Ganges delta¹¹—16–17 km). A considerable length of the Atlantic continental margin of North America is mantled by a sediment prism 10–12 km thick¹⁰. Reconstructed ancient geosynclinal sediment thicknesses are also commonly in this range¹².

Thus the sediment loading capacities of rifted continental margins, the maximum age of rifted margins and the rate of delivery of sediment from the craton may all lead to massive sediment prisms being developed along rifted continental margins. The oldest oceanic crust and the continent-ocean transition zone, together with the earliest sediments deposited in the proto-ocean, typically become very deeply buried.

Continental margin and ocean floor evaporites

Bathymetric and geophysical surveys of certain continental slopes and rises and of adjacent deep oceanic areas give abundant evidence of the presence of diapiric structures. A variety of arguments suggests that most of these are evaporite

Fig. 1 Subsidence histories of ocean floor as a function of post-rifting age. (a), Increase in water loading of oceanic crust as a function of post-rifting age (modified from Sclater and Francheteau⁴); (b), maximum ocean floor loading with halite as a function of post-rifting age; (c), maximum, isostatic, marine sediment loading of ocean floor as a function of post-rifting age (from Kinsman⁶).



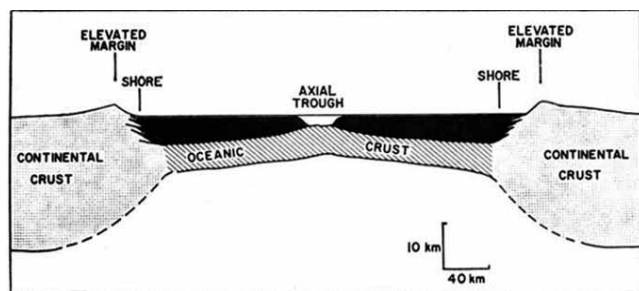


Fig. 2 Schematic depicting proto-ocean evaporites in the southern Red Sea (adapted from data in Hutchinson and Engels¹⁶ and Whitmarsh *et al.*¹⁷). The upper 3–4 km of the evaporites are proved by drilling, the lower section from seismic information; data available mainly from the western margin of the Red Sea.

diapirs rather than igneous intrusions or mud diapirs¹³. In a few instances drilling has recovered typical salt dome cap rock, as on the Sigsbee Knolls of the central Gulf of Mexico¹⁴. In other instances pore waters above the diapiric structures have proved to be very saline, indicating the deeper presence of halite¹⁵.

Hutchinson and Engels¹⁶ and Whitmarsh *et al.*¹⁷ provide information on the Red Sea continental margin and proto-ocean evaporites; the marginal and proto-ocean evaporites from the South Atlantic have most recently been described by Pautot *et al.*¹⁸; the North Atlantic continental margin evaporite data have been summarised by Emery and Uchupi¹⁰. These three oceans are all rift oceans in various stages of development; the Red Sea is a proto-ocean in an early stage of development (~20 Myr), the South Atlantic is a mature rift ocean (~100–120 Myr), and the North Atlantic is entering its probable senescence as a rift ocean (~160–200 Myr). In all three oceans the evaporites are recorded in marginal basins, down the continental slope and on oceanic crust. For example, in the South Atlantic diapirs are reported seaward of the Niger Delta¹⁹ in an area which is surely underlain by oceanic crust as it comprises an overlap zone in all pre-rift reconstructions. The lateral extent of the evaporites is such that salt flowage or creep seaward down the continental slope and across the adjacent ocean floor seems a most unlikely explanation of the diapirs overlying oceanic crust. The detailed pattern of evaporite deposition in and along the margins of the proto-ocean may have been very complex. For example, local evaporite formation may proceed in marginal grabens or basins such as the 1-km thick Pleistocene evaporites of the Danakil Depression¹⁶ which were formed while the main Red Sea proto-ocean was in a non-evaporite mode. At other times the entire proto-ocean may become partially or completely isolated and the brine–air interface may exist anywhere between world ocean level and the floor of the proto-ocean basin 2–3 km below. The evaporites may therefore show the characteristics of shallow water or even subaerial sabkha facies²⁰, or be well layered deep brine basin deposits.

The presence of thick evaporites in rift valley basins and in proto-oceans suggests that times of initiation of new continental rifting and proto-ocean formation may be major evaporite formation periods. The widths of these continental margin evaporite belts, when combined with probable spreading rates, suggest that evaporite formation occurs during the initial 10–20 Myr of spreading^{6,21}. The distribution of the Red Sea evaporite thicknesses and the age of the Red Sea conform fairly closely to the predicted maximum salt loading curve for oceanic crust (Fig. 1). The evaporites are about 3 km thick immediately west of the present axial trough (Fig. 2) and thicken westward to a maximum of about 7 km (ref. 16). This landward thickening suggests that the evaporites are overlying oceanic crust which increases in age westward. The evaporite and sediment section thins again westward of the maximum

7 km evaporite zone, presumably because of the gradual westward thickening of continental crust (Fig. 2).

If the future history of the Red Sea continental margins is to be similar to that recorded for most ancient rifted continental margins, subsidence will depress the entire belt of thinned continental crustal margin, the adjacent coupled oceanic crust and the overlying evaporite body. Normal marine sediments more than 10 km thick, will probably be deposited on top of the evaporites. The occurrence of continental margin evaporites in the South Atlantic (~120–110 Myr) and North Atlantic (~200–160 Myr) can be reasonably understood if these oceans underwent an early history similar to that of the Red Sea, with thick evaporites laid down over the entire continental margin and oldest ocean floor belt (Fig. 3).

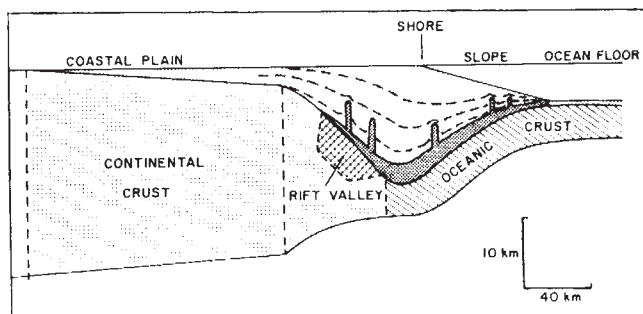
Widths of the continental margin evaporites vary greatly and along some local lengths of margin evaporites may be absent. Evaporites are often present in minor rift basins but are absent over local structural highs. Offshore data are generally too sparse to be certain of the detailed evaporite distribution. But in the Red Sea the belt of evaporites is up to 100 km wide along the south-west margin. In the South Atlantic the belt of evaporites in the coastal basins and extending down the continental slope to the abyssal plain is 50–300 km wide¹⁸; similar widths are reported off the Brazilian coast²². In the North Atlantic, diapirs have been reported 1,300 km off the north-west African coast²³, but these are not yet proven to be evaporites. The Gulf of Mexico evaporites also cover a very wide area. Thus the widths of rifted continental margin evaporite belts are such that most of the future eugeosynclinal sediment prism will be deposited on top evaporitic sediments. Only along continental margins where sediment input rates are very high may the width of the evaporite belt be overstepped by the thick prism of sediments.

Frequency of evaporite formation

How frequently are evaporites formed in proto-oceans? To form an evaporite deposit two independent requirements must be met.

First, the proto-ocean must be located in a climatic belt of net evaporation. Present-day marine evaporites are largely restricted to the latitudes 30–35° N or S of the Equator, although a few occurrences extend to 40° N or S. Smith *et al.*²⁴ and Drewry *et al.*²⁵ have published a series of maps showing the likely predrift configurations of the continents and their palaeolatitudinal positions for various past geological periods. One of their major conclusions, based on the distribution of marine evaporites and other climatically sensitive sediments is that since at least late Mesozoic times the high pressure subtropical belts of net evaporation have had approximately their present-day width and have been fairly stable features of the atmospheric circulation. It seems reasonable, therefore, to assume that nearly all evaporites over the past 200 Myr have formed between the latitudes 35°N and 35°S.

Fig. 3 Schematic depicting proto-ocean evaporites deeply buried at the base of a trailing continental margin sediment prism (modified from Fig. 10 of ref. 6). This section is similar in most respects to the probable north south section across the Mississippi Delta and northern Gulf of Mexico.



Second, proto-oceans within the climatic belt of net evaporation must develop sufficient physical restriction to seawater influx. The restriction requirement is extremely demanding as the seawater influx must be less than the total evaporation, and this latter parameter is climatically constrained to values of less than $2\text{--}3\text{ myr}^{-1}$. The barriers restricting seawater influx to proto-oceans comprise the elevated margins of the rifted continents along the length of the proto-ocean, together with transverse barriers which may be continental fragments faulted off the main continental margin, continental segments where rifting is transcurrent, or volcanic islands over deep mantle plume sites^{6,21}.

The maps of Smith *et al.*¹⁹ together with additional data on age of rifting in various localities and on presence or absence of evaporites along the rifted continental margins have been used to generate Table 1, *a* and *b*.

Of the rift basins and proto-oceans in which evaporites were formed only the Tanzania example is at a latitude higher than 30° (Table 1*a*). The total axial length of proto-ocean generated over the past 200 Myr between latitudes 35°N and 35°S is approximately 11,000 km and in 9,000 km evaporites were formed on a massive scale (1–7 km thick). Thus, rather than being an unusual phenomenon, evaporites are the expected and predictable sediments to form in low latitude proto-oceans during their initial 10–20 Myr of development. There is an 80–85% probability of the physical barrier requirement being met and this is a purely geological parameter independent of the climatic requirements.

A rough estimate of the proto-ocean formed at latitudes higher than 35° over the past 200 Myr is 14,500 km (Table 1*b*). During this period of 200 Myr nearly 35% of the total proto-ocean length has been deeply mantled by an early deposit of evaporites. In other words, 35% of potential eugeosynclinal floors formed over the past 200 Myr are underlain by thick and laterally extensive evaporite deposits.

A corollary of the frequency with which low latitude proto-oceans develop a physical restriction sufficient for them to become evaporitic is that in latitudes of net precipitation, the majority (~80%) of proto-oceans should have been brackish during their initial 10–20 Myr of post rifting development.

The climatic belt in which evaporites may form comprises

approximately half the area of the Earth. If the frequency of achievement of physical restriction to seawater influx into proto-oceans is about 80% and if proto-oceans have been generated throughout the Earth's history with no latitudinal bias, then about 40% of all rifted continental margins should have been mantled with evaporites. If all eugeosynclines have gone through an early trailing continental margin stage then 40% of the axial length of all eugeosynclinal floors should have been mantled by evaporites.

Consequences of proto-ocean evaporite formation

There are several interesting consequences to the proposal that evaporites very commonly form in proto-oceans yet later line the floors of geosynclines:

Evaporite masses formed in proto-oceans are commonly very large (500–4,000 km long, 100–600 km wide and 1–7 km thick) and the period of formation is relatively short (<10–20 Myr). Compared with the rate of input of Na^+ and Cl^- into the ocean²⁶, the evaporite withdrawal of NaCl as halite is essentially instantaneous and will produce a salinity decrease of the world ocean. Estimates of NaCl stored in evaporites are certainly too small and are in need of drastic upward revision (my unpublished work); when these data are available reasonable estimates can be made of the evaporite controlled salinity excursions of the world ocean. If these salinity excursions are large enough to be reflected in the biotic record, then a link may be established between times of large scale rifting and inception of seafloor spreading and biotic crises, through the evaporite formation mechanism.

The deep burial of evaporites beneath thick sediment prisms opens an interesting avenue to the problem of the sodium cycle. The weathering of crustal silicate rocks releases sodium to the ocean and forms a sodium depleted sediment. With passage of time, considerable burial, or moderate temperature increase, there is, however, little or no marked accession of sodium to sediments^{27,28}. Even low grade metamorphosed sedimentary rocks such as mica schists are still sodium depleted. Higher grade feldspar gneisses, however, have sodium values typical of average continental crustal rock²⁸. These relationships suggest that halite dominated evaporites are subducted together with

Table 1 Proto-oceans and continental margins

<i>a</i> With evaporites			
Proto-Ocean/Continental Margin*	Rifting/evaporite age (Myr)†	Palaeolatitude‡	Axial length (km)§
Red Sea + Danakil Depression	25–0 (U. Oligo–Rec)	5°N – 30°N	2,000
South Atlantic	120–110 (Aptian–L. Albian)	10°S – 30°S	2,500
Tanzania	220–170 (U. Trias–M. Jur.)	35°S	500
N. Atlantic + Gulf of Mexico	200–160 (U. Trias–M. Jur.)	5°N – 25°N	4,000
		Total	9,000
<i>b</i> Without evaporites			
Gulf of California	~5 (Plio.–Rec.)	20°N – 30°N	1,200
Gulf of Aden	15–10 (Mio.–Plio.)	5°N – 10°N	800
		(Low latitude subtotal)	2,000
Australia–Antarctica	~50 (Eoc.)	60°S – 65°S	2,500
N. Atlantic–Norway, Greenland	~60 (U. Palaeoc.–L. Eoc.)	45°N – 60°N	2,500
Baffin Bay	~60 (U. Palaeoc.–L. Eoc.)	45°N – 60°N	1,500
Australia–New Zealand	80–60 (U. Cret.–Palaeoc.)	50°S – 70°S	1,000
India–Antarctica	~125 (L. Cret.)	40°S – 50°S	1,500
West Australia–India	~125 (L. Cret.)	40°S – 50°S	1,000
Africa–Madagascar } Madagascar–India }	~140 (U. Jur.–L. Cret.)	35°S – 50°S	3,000
NW Australia–?	~150 (U. Jur.)	40°S – 50°S	1,500
		Total	16,500

* Proto-oceans are identified where possible by use of the ocean name, or by use of the two bounding rifted continental margins (for example, Africa–Madagascar); where only one rifted margin name appears, we have no evidence of the opposing margin. The Tanzania evaporites were apparently formed in a marginal rift graben; no evaporites were formed in the associated proto-ocean.

† Rifting/evaporite age data are from refs 7, 10, 17, 18, 31–34.

‡ Palaeolatitude data are derived from using age data of previously mentioned authors in conjunction with maps of Smith *et al.*²⁴. The large latitudinal range of Africa–Madagascar–India in part reflects the uncertainty of the palaeoposition of Madagascar relative to the African continent.

§ Axial length refers to proto-ocean length (about twice this length of rifted continental margin has been formed over the past 200 Myr).

sodium depleted sediments and that sodium metasomatism occurs during metamorphism. The halite will penetrate the sediment mass partly in the anhydrous state as diapirs and partly in solution, being dissolved and mobilised by remnant pore water and structural water removed from hydrated minerals during dehydration reactions. A sediment column 15 km thick would need to react with only about 200 m of halite to restore its sodium content to that of average crustal rock. Recent reports of NaCl brines in fluid inclusions from high grade metamorphic rocks may support this proposal²⁹. One might predict that if such solutions gained their Na⁺ and Cl⁻ from halite dissolution then the Br⁻/Cl⁻ ratios should be much lower than that of seawater.

Halite deposits high on the continental margin presumably will not be subducted. These could form a surface of detachment enabling some of the compressed sediment prism to ride more readily up on to the continental margin.

The continental slopes are becoming of economic interest. Many of the world's hydrocarbon reservoirs are sealed by evaporites because they have essentially zero porosity and permeability. Are there any likely hydrocarbon source beds beneath the extensive evaporites of early rift oceans? If the evaporites form so early in the history of the ocean one might surmise the probability to be small. But the immediately pre-evaporite phase of restriction, leading to hypersalinity is one in which stable stratification of water bodies may be expected. In addition, dissolved molecular oxygen in the seawater brines is low because of relatively high water temperatures (20–30 °C) and increasing salinity³⁰. As yet, we know little about organic productivity in evaporite basins but it seems likely that much of the organic matter which is produced will be sedimented and not oxidised. There is a fair possibility that early rift oceans with evaporites could have had organic rich sediments accumulate within them previous to evaporite deposition. Hydrocarbons generated from these sediments would be sealed beneath the

overlying evaporites and might occur in marginal reservoir facies along the continental margin.

I thank M. Arthur, K. S. Deffeyes, A. G. Fischer, R. B. Hargraves, F. B. Van Houten and R. Yuretech for comments. These studies were largely completed during my tenure of a Guggenheim Memorial Fellowship.

Received March 17; accepted April 14, 1975.

- ¹ Hess, H. H., *Bull. geol. Soc. Am.* **71**, 235–240 (1960).
- ² Dietz, R. S., *J. Geol.* **71**, 314–333 (1963).
- ³ Dietz, R. S., *Am. J. Sci.*, **264**, 177–193 (1966).
- ⁴ Sclater, J. S., and Francheteau, J., *Geophys. J. R. astr. Soc.*, **20**, 509–542 (1970).
- ⁵ Morgan, W. J., *Bull. Am. Assoc. petrol. Geol.*, **56**, 203–213 (1972).
- ⁶ Kinsman, D. J. J., in *Petroleum and Global Tectonics* (edit. by Fischer, A. G., and Judson, S.), 84–126, (Princeton Univ. Press, 1975).
- ⁷ Vogt, P. R., and Avery, O. E., *J. geophys. Res.*, **79**, 363–389 (1974).
- ⁸ Sleep, N. H., *Geophys. J. R. Astr. Soc.*, **24**, 325–350 (1971).
- ⁹ Hospers, J., *Bull. geol. Soc. Am.*, **76**, 407–422 (1965).
- ¹⁰ Emery, K. O., Uchupi, E., *Am. Ass. petrol. Geol. Memoir*, **17** (1972).
- ¹¹ Moore, D. G., Curry, J. T., and Raitt, R. W., *Abstr. VIII int. Sed. Cong.*, **69** (Heidelberg, 1971).
- ¹² Stonely, R., *Geol. Soc. London Spec. Publ.*, **3**, 215–238 (1969).
- ¹³ Schneider, E. D., and Johnson, G. L., *Bull. Am. Ass. petrol. Geol.* **54**, 2151–2169 (1970).
- ¹⁴ Burk, C. A., et al., *Bull. Am. Ass. petrol. Geol.*, **53**, 1338–1347 (1969).
- ¹⁵ Manheim, F. T., Sayles, F. L., in *The Sea*, **5** (edit. by Goldberg, E. D.), 527–568 (Wiley-Interscience, New York and London, 1974).
- ¹⁶ Hutchinson, R. W., Engels, G. G., *Bull. Geol. Soc. Am.*, **83**, 2989–3002 (1972).
- ¹⁷ Whitmarsh, R. B., et al., *Init. Rep. Deep Sea Drilling Project*, **23** (Washington, 1974).
- ¹⁸ Pautot, G., et al., *Bull. Am. Ass. petrol. Geol.*, **57**, 1658–1671 (1973).
- ¹⁹ Mascle, J. R., et al., *Bull. Am. Ass. petrol. Geol.*, **57**, 1672–1678 (1973).
- ²⁰ Kinsman, D. J. J., *Bull. Am. Ass. petrol. Geol.*, **53**, 830–840 (1969).
- ²¹ Kinsman, D. J. J., in *Proc. IV Salt Symp.* (edit. by Coogan, A. H.), 255–260 (Northern Ohio Geol. Soc. 1974).
- ²² Leyden, R., et al., *Bull. Am. Ass. petrol. Geol.*, **55**, 2161–2173 (1971).
- ²³ Rona, P. A., *Nature*, **224**, 141–143 (1969).
- ²⁴ Smith, A. G., et al., in *Palaeont. Assoc. Spec. Paper*, **12** (edit. by Hughes, N. F.), 1–42 (1973).
- ²⁵ Drewry, et al., *J. Geol.*, **82**, 531–553 (1974).
- ²⁶ Garrels, R. M., and Perry, E. A., in *The Sea*, **5** (edit. by Goldberg, E. D.), 303–336 (Wiley-Interscience, New York and London, 1974).
- ²⁷ Livingstone, D. A., *Geochim. cosmochim. Acta*, **27**, 1055–1069 (1963).
- ²⁸ Garrels, R. M., and MacKenzie, F. T., *Evolution of Sedimentary Rocks* (Norton, New York, 1971).
- ²⁹ Rich, R. A., *Geol. Soc. Am. Abst.*, **5**, 780–781 (1973).
- ³⁰ Kinsman, D. J. J., et al., in *Proc. IV Salt Symp.* (edit. by Coogan, A. H.), 325–328 (Northern Ohio Geol. Soc. 1974).
- ³¹ Reymont, R. A., and Tait, E. A., *Phil. Trans. R. Soc.*, **264**, 55–95 (1972).
- ³² Kent, P. E., in *Salt Basins Around Africa*, 41–54 (Institute of Petrol., London, 1965).
- ³³ Larson, R. L., et al., *Science*, **161**, 781–784 (1968).
- ³⁴ Smith, A. G., and Hallam, A., *Nature*, **225**, 139–144 (1970).

Induction of increased calcium uptake in mouse T lymphocytes by concanavalin A and its modulation by cyclic nucleotides

Murray H. Freedman & Martin C. Raff

MRC Neuroimmunology Project, Department of Zoology, University College London, London WC1E 6BT, UK

Bastien Gomperts

Department of Experimental Pathology, University College Hospital Medical School, London, WC1E 6JJ, UK

The binding of concanavalin A to T but not B mouse spleen lymphocytes increases Ca²⁺ uptake in these cells which is measurable by 45 s and complete by 1 min. Dibutyryl cyclic AMP, but not sodium azide inhibits induced Ca²⁺ uptake, whereas dibutyryl cyclic GMP enhances it. B cell mitogens do not cause a similar Ca²⁺ uptake in mouse B lymphocytes. The induction of increased Ca²⁺ uptake by T cells is discussed in terms of gated membrane channels for Ca²⁺.

CELL surface receptors receive and transduce a variety of environmental signals. The binding of such a signalling ligand (or 'first messenger') induces a change in the receptor, initiating the next event(s) by which information is carried across the

membrane into the cell interior. The cyclic nucleotides, cyclic AMP and cyclic GMP, which are regulated by specific membrane bound enzymes (cyclases), have received much attention as 'second messengers' in the signalling of cellular activity. It is clear, however, that in some situations it is the opening of ion permeability pathways through membranes which serves this function by allowing the transfer of selected ions, such as Na⁺ and Ca²⁺, down their concentration gradients into the cytosol. Thus, Ca²⁺ is the second messenger in excitation-contraction coupling in skeletal muscle¹ and probably plays a similar role in the 'stimulus-secretion coupling' of many triggered secretory systems². There is increasing indirect evidence that is consistent with a role for Ca²⁺ as a transducing mediator in more prolonged responses, such as the lectin-induced proliferation of lymphocytes. These responses are dependent on the presence of Ca²⁺ in the extracellular medium^{3–5}, and an increased influx of radioactive ⁴⁵Ca²⁺ has been

Table 1 Measuring and calculating $^{45}\text{Ca}^{2+}$ uptake*

Mitogen	Time of incubation (min)	$^3\text{H}_2\text{O}$ (c.p.m.)	$^{45}\text{Ca}^{2+}$ (c.p.m.)	$^{45}\text{Ca}^{2+}/^3\text{H}_2\text{O}$	$^{45}\text{Ca}^{2+}$ uptake	Corrected§ $^3\text{H}_2\text{O}$ (c.p.m.)	Corrected $^{45}\text{Ca}^{2+}$ (c.p.m.)	Corrected $^{45}\text{Ca}^{2+}/^3\text{H}_2\text{O}$	Corrected $^{45}\text{Ca}^{2+}$ uptake
None†	0	1,478 ± 62	760 ± 33	0.514 ± 0.01	—	—	—	—	—
None	15	1,325 ± 80	948 ± 34	0.719 ± 0.03	1.0 ± 0.036	869	188	0.216	1.0
Con A (3 µg ml ⁻¹)	15	1,889 ± 15	2,110 ± 23	1.117 ± 0.003	1.55 ± 0.004	1,433	1,350	0.942	4.36
A23187 (2 × 10 ⁻⁵ M)	15	1,446 ± 92	2,993 ± 143	2.066 ± 0.05	2.87 ± 0.07	990	2,233	2.255	10.49

* Results of a typical experiment done in triplicate, expressed as mean ± s.d.; cells, mitogens and isotopes incubated as in text, or as indicated.

† Chilled $^{45}\text{Ca}^{2+}$ and $^3\text{H}_2\text{O}$ added to cells at 4 °C and centrifuged immediately; $^{45}\text{Ca}^{2+}$ counts are mainly due to exchange with surface-bound cations and radioactivity in entrained water space (see text).

‡ $^{45}\text{Ca}^{2+}$ uptake is calculated by dividing the $^{45}\text{Ca}^{2+}/^3\text{H}_2\text{O}$ value of the sample by that of the control cells incubated with MEM alone, so that control ratio is normalised to 1.0. The increase above 1.0 is a measure of the mitogen-induced $^{45}\text{Ca}^{2+}$ uptake.

§ Corrected by subtracting the 0.15% of the $^3\text{H}_2\text{O}$ load (= 456 c.p.m. in this experiment) which is in the entrained water space (see text).

|| Corrected by subtracting the $^{45}\text{Ca}^{2+}$ counts at time 0 at 4 °C (= 760 c.p.m.).

reported to occur within minutes of PHA binding to human blood lymphocytes⁶⁻⁸. It has not so far been demonstrated, however, that the cells showing increased permeability for Ca^{2+} are those which are later induced to divide. Recently, pig⁹ and human¹⁰ lymphocytes have been induced to synthesise DNA by application of the divalent cation-carrier molecule (ionophore) A23187, in a manner dependent on the presence of extracellular Ca^{2+} . This suggests that the influx of Ca^{2+} may be a sufficient signal for proliferation. So far, rodent lymphocytes have not been reported to respond to the ionophore, and except for one preliminary experiment⁷, there are no substantive reports of Ca^{2+} influx occurring as a consequence of the binding of lectins to these cells. Here we report that concanavalin A (con A) induces increased $^{45}\text{Ca}^{2+}$ uptake in mouse T, but not B lymphocytes within 45 s of the addition of lectin. This effect is short lived, and is inhibited by high concentrations of dibutyl cyclic AMP but not by sodium azide, and it is enhanced by dibutyl cyclic GMP. In addition, we show that two B lymphocyte mitogens do not cause a measurable increase in $^{45}\text{Ca}^{2+}$ in B cells. These findings raise important questions about the possible role of gated Ca^{2+} channels in ligand-induced lymphocyte proliferation.

Measuring $^{45}\text{Ca}^{2+}$ uptake

BALB/c mouse spleens were teased in Eagle's medium (containing 1.8 mM Ca^{2+}), buffered with HEPES 20 mM (instead of HCO_3^-) to pH 7.2 (MEM), passed over a short column of glass wool to remove debris and cell clumps, treated with Tris-buffered ammonium chloride pH 7.2, for 10 min at room temperature to lyse red cells¹¹, washed twice, and adjusted to 20×10^6 – 30×10^6 cells ml⁻¹. Cells (80 µl) were incubated with 10 µl of con A (Miles-Yeda) or MEM, and 10 µl of a mixture of $^{45}\text{CaCl}_2$ (1 µCi) and $^3\text{H}_2\text{O}$ (5 µCi). The equilibration of water across phospholipid and biological membranes at 37 °C is very rapid¹², allowing the uptake of tritiated water in these experiments to serve as an indicator of cell number. After 15 min at 37 °C, the cells were separated from the radioactive loading solution by centrifugation at 12,000g for 3 min through a water impermeable barrier of silicone oil (Versilube F50) into 20 µl of 98% formic acid. Polypropylene microcentrifuge tubes (Beckman PRO-22) and a Beckman 152 Microfuge were used for this purpose. After centrifugation, the contents of the tubes were frozen by immersion in liquid nitrogen, and the bottom 1 cm, containing the cells now dissolved in formic acid, was amputated. This was placed in 3 ml of Instagel (Packard) and counted in a Packard Tricarb liquid scintillation spectrometer. The ratio of $^{45}\text{Ca}/^3\text{H}$ counts is proportional to the uptake of $^{45}\text{Ca}^{2+}$ per cell.

When ^{51}Cr -labelled spleen cells were handled in this way, 85–90% of the radioactivity was transferred into the formic acid, indicating that the great majority of the cells were centrifuged through the silicone oil. By adding ^3H -sucrose to the cell suspensions as a marker of entrained water space it

was demonstrated that 0.15% of the volume of the loading solution was transferred through the silicone layer with 3×10^6 cells (that is 0.15 µl). When chilled $^{45}\text{Ca}^{2+}$ was added to cells incubated in MEM at 4 °C and immediately centrifuged, a relatively large uptake of activity was consistently seen, amounting to about 0.4% of the total $^{45}\text{Ca}^{2+}$ in the loading solution, but representing about 60–80% of the $^{45}\text{Ca}^{2+}$ uptake occurring when cells were incubated for 15 min at 37 °C (Table 1). Thus the major part of the $^{45}\text{Ca}^{2+}$ associated with the cells in these experiments probably represented rapid exchange of the labelled material with the cations adsorbed to the cell surface and was not related to membrane transport.

Table 2 Effect of mitogens on $^{45}\text{Ca}^{2+}$ uptake in BALB/c spleen lymphocytes, human RBC and mouse lymphoma cells*

Cells	Mitogen†	Concentration (µg ml ⁻¹)	No. of experiments	Uncorrected‡ $^{45}\text{Ca}^{2+}$ uptake
Spleen	None	—	—	1.0
Spleen	Con A	3	31	1.30 ± 0.034
Spleen	PHA	4	7	1.22 ± 0.034
Spleen	Pokeweed	15	1	1.39 ± 0.052
Spleen	Axinella	10	3	1.00 ± 0.012
Spleen	LPS	20	17	0.98 ± 0.011
Spleen	A23187	2 × 10 ⁻⁵ M	17	3.09 ± 0.156
Spleen	A23187	2 × 10 ⁻⁶ M	1	2.04 ± 0.012
RBC§	None	—	—	1.0
RBC	Con A	4	1	0.95 ± 0.004
RBC	LPS	20	1	0.90 ± 0.001
RBC	A23187	2 × 10 ⁻⁵ M	3	13.02 ± 0.304
WEHI-22	None	—	2	1.0
WEHI-22	Con A	3	2	0.98 ± 0.020
WEHI-22	Con A	50	2	1.55 ± 0.061
WEHI-22	Con A	100	2	1.95 ± 0.066
EL-4¶	None	—	1	1.0
EL-4	Con A	3	1	1.01 ± 0.031

* Cells, isotopes and mitogens were incubated at 37 °C for 15 min; each experiment was done in triplicate.

† Con A = 3 × crystallised concanavalin A (Miles-Yeda); PHA = purified *P. vulgaris* phytohaemagglutinin (Wellcome); pokeweed = pokeweed (*Phytolacca americana*); mitogen was a gift from Dr G. Janossy and was prepared as described by Borjeson *et al.*¹⁴; LPS = lipopolysaccharide from *salmonella marcescens* (Difco); Axinella = *Axinella polyloides* agglutinin, was a gift from Dr M. Crumpton, and was prepared as previously described¹⁵; A23187 was supplied by Dr R. L. Hamill (Eli Lilly). Con A, PHA, pokeweed, and LPS were used at concentrations which gave maximum DNA synthetic responses in spleen cells at 72 h.

‡ Determined as in Table 1 and expressed as mean ± s.d. of the means of each experimental triplicate. Where only 1 experiment was done, the mean ± s.d. of the triplicate is given. Positive responses are underlined.

§ Human RBC were collected in heparinised MEM, washed twice and used at about a 5% suspension.

|| WEHI-22 is a cultured cell line from an irradiation-induced BALB/c θ⁺ lymphoma¹⁷.

¶ EL-4 is a cultured cell line from a chemically-induced C57B1 θ⁺ thymoma¹.

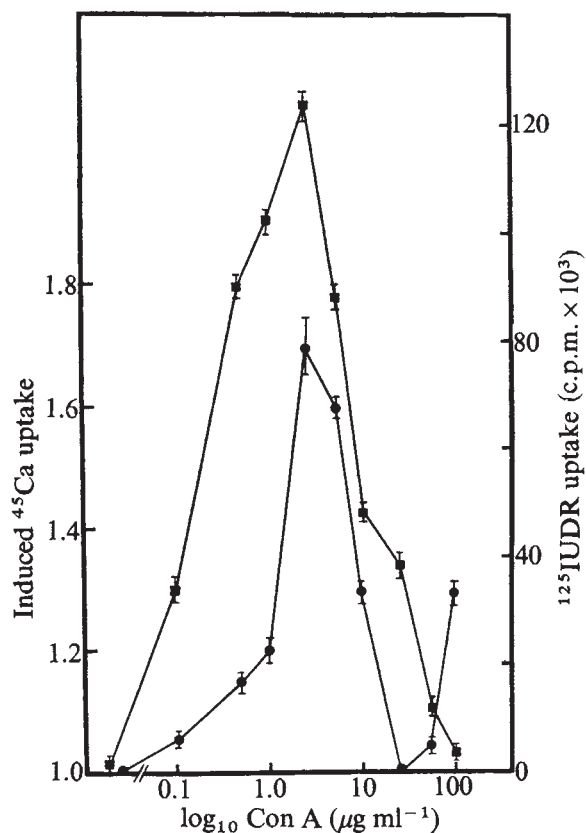


Fig. 1 Dose responses of uncorrected Con A-induced $^{45}\text{Ca}^{2+}$ uptake (●) measured at 15 min (as in Table 1) and DNA synthesis (■) measured by ^{125}I UDR incorporation at 72 h. The same BALB/c spleen suspension at 30×10^6 cells ml^{-1} in RPMI-1640 (buffered with bicarbonate) medium plus 10% foetal calf serum was used for both assays. For ^{125}I UDR assay, 200 μl of cells were incubated with con A for 60 h in Limbro Microtest plates at which time 1 μCi in ^{125}I UDR was added to each well for another 12 h. The cells were collected on to Whatman GF/A filter paper, washed with water and counted in a Wallac γ -counter. In both assays, results are expressed as means $\pm$ s.d. of triplicates.

$^{45}\text{Ca}^{2+}$ uptake increased by T cell mitogens

Spleen cells exposed to appropriate concentrations of known T lymphocyte mitogens¹³, including con A, phytohaemagglutinin (PHA) and pokeweed mitogen, showed measurable increases in $^{45}\text{Ca}^{2+}$ uptake compared with cells incubated in MEM (Tables 1 and 2). When $^{45}\text{Ca}^{2+}/^3\text{H}_2\text{O}$ ratios were compared, these increases varied between 10 and 80%. If the 60–80% of the $^{45}\text{Ca}^{2+}$ counts present at the cell surface and within the entrained water space were subtracted, however, the increases were 150–500% (Table 1). Except for Table 1, all of the data are presented as uncorrected ratios.

Spleen cells treated with various concentrations of lipopolysaccharide from *Salmonella marcescens* (LPS)¹³ or *Axinella polyptoides* agglutinin¹⁵, both of which are known not to be mitogenic for mouse T cells, failed to show increased $^{45}\text{Ca}^{2+}$ uptake. A23187, the divalent cation ionophore¹⁶, permitted spleen cells to increase their $^{45}\text{Ca}^{2+}/^3\text{H}_2\text{O}$ ratio 2–4-fold (Table 2). Human red cells and cell lines derived from two θ^+ mouse lymphomas, WEHI-22¹⁷ and EL-4¹⁸, did not show an increase in $^{45}\text{Ca}^{2+}$ uptake when exposed to mitogenic (for T cells) concentrations of con A, but WEHI-22 cells did when exposed to very high concentrations of the lectin (Table 2).

The dose responses for con A-induced $^{45}\text{Ca}^{2+}$ uptake and DNA synthesis (measured by the ^{125}I UDR incorporation method at 72 h) determined for the same spleen cell suspension in identical conditions of incubation are shown in Fig. 1. At the lower concentration end, the dose-response curves were very similar, with a sharp peak at about $3 \mu\text{g ml}^{-1}$. Although no DNA synthesis was seen with concentrations of con A

greater than $25 \mu\text{g ml}^{-1}$, the uptake of $^{45}\text{Ca}^{2+}$ increased again at these high concentrations. Marked agglutination and sticking of the cells to the walls of the test tube occurred at these high concentrations of con A, making it difficult to interpret this second rise in $^{45}\text{Ca}^{2+}$ uptake. No appreciable agglutination of the spleen cells occurred at concentrations of con A $\leq 3 \mu\text{g ml}^{-1}$.

Only T cells respond

Although soluble con A binds to all mouse spleen cells and the same number of molecules binds with equal avidity to both T and B lymphocytes¹⁹, only T cells are stimulated to proliferate¹³. To find out if the con A-induced $^{45}\text{Ca}^{2+}$ uptake was also restricted to the T lymphocytes, spleen cells were treated with anti- θ C3H (anti-thy-1.1) antiserum and guinea pig complement to kill the T cells²⁰; control cells were treated with normal mouse serum and complement. In both cases, dead cells were removed, following treatment, by a 5-min exposure to 0.05% trypsin at 37 °C. The control cells (containing 36% T cells as judged by direct immunofluorescence with highly specific fluorescein-conjugated anti- θ C3H²¹) showed a 30% increase in $^{45}\text{Ca}^{2+}$ uptake in the presence of con A, while the cells which had been treated with anti- θ antibody and complement (less than 1% T cells) failed to show any increment (Table 3). Spleen cells from congenitally hypothyroid *nu/nu* (nude) mice, known to be markedly deficient in T cells²¹, failed to show an increase in $^{45}\text{Ca}^{2+}$ uptake on exposure to mitogenic concentrations of con A, but did so at very high concentrations (Table 3). Thus the increase in the uptake of $^{45}\text{Ca}^{2+}$ at mitogenic concentrations of con A depended on the presence of T cells, whereas that seen at high concentrations ($> 10 \mu\text{g ml}^{-1}$) did not.

Although mouse spleen B lymphocytes are induced to proliferate by bacterial lipopolysaccharide (LPS)¹³, no increase

Table 3 Effect of mitogens on $^{45}\text{Ca}^{2+}$ uptake in T-cell-depleted spleen cells

Cells	Treatment* of cells	Mitogen	Concentration (μg ml ⁻¹)	No of experiments	Uncorrected† $^{45}\text{Ca}^{2+}$ uptake
BALB/c spleen	anti- θ	none	—	—	—
BALB/c spleen	anti- θ	Con A	0.5	2	<u>0.95 ± 0.006</u>
BALB/c spleen	anti- θ	Con A	3.3	2	<u>0.99 ± 0.011</u>
BALB/c spleen	anti- θ	Con A	5.0	2	<u>0.97 ± 0.027</u>
BALB/c spleen	NMS	none	—	—	—
BALB/c spleen	NMS	Con A	0.5	2	<u>1.09 ± 0.017</u>
BALB/c spleen	NMS	Con A	3.3	2	<u>1.30 ± 0.033</u>
BALB/c spleen	NMS	Con A	5.0	2	<u>1.22 ± 0.025</u>
<i>nu/nu</i> spleen‡	—	none	—	—	1.00
<i>nu/nu</i> spleen	—	Con A	0.5	6	<u>1.01 ± 0.02</u>
<i>nu/nu</i> spleen	—	Con A	3.3	6	<u>0.98 ± 0.02</u>
<i>nu/nu</i> spleen	—	Con A	5.0	6	<u>0.98 ± 0.013</u>
<i>nu/nu</i> spleen	—	Con A	10.0	6	<u>1.00 ± 0.008</u>
<i>nu/nu</i> spleen	—	Con A	50.0	3	<u>1.55 ± 0.11</u>
<i>nu/nu</i> spleen	—	Con A	100.0	3	<u>1.80 ± 0.25</u>
<i>nu/nu</i> spleen	—	LPS§	10.0	4	<u>0.99 ± 0.01</u>
<i>nu/nu</i> spleen	—	LPS§	30.0	4	<u>1.01 ± 0.017</u>
<i>nu/nu</i> spleen	—	LPS§	50.0	4	<u>1.03 ± 0.02</u>
<i>nu/nu</i> spleen	—	Pokeweed§	5.0	3	<u>0.96 ± 0.025</u>
<i>nu/nu</i> spleen	—	Pokeweed§	15.0	3	<u>1.01 ± 0.006</u>
<i>nu/nu</i> spleen	—	Pokeweed§	25.0	3	<u>0.96 ± 0.015</u>

* 200 $\times 10^6$ cells were treated with 2 ml of anti- θ C3H or normal mouse serum (NMS) diluted 1:4 in MEM for 30 min at 37 °C, washed and incubated with 2 ml of guinea pig complement (absorbed with mouse spleen cells) at 1:4 for 30 min at 37 °C. After washing, cells were treated with 2 ml of 0.05% trypsin in MEM for 5 min at 37 °C to remove dead cells and washed twice. Anti- θ -treated cells contained 0.8% T cells as judged by direct immunofluorescence with anti- θ C3H-fluorescein, while NMS-treated cells contained 36% T cells. Cells were tested as described in text.

† Determined as in Table 1 and expressed as mean $\pm$ s.d. of each experimental triplicate. Positive responses are underlined.

‡ *nu/nu* mice were bred at the Clinical Research Centre, Harrow, and where supplied by Dr E. Simpson.

§ When used at these concentrations, these preparations stimulated *nu/nu* spleen cells to synthesise DNA (tested by Dr G. Janossy).

in $^{45}\text{Ca}^{2+}$ uptake could be detected when BALB/c or *nu/nu* spleen lymphocytes were treated with mitogenic concentrations of this B cell mitogen (Tables 2 and 3). Pokeweed mitogen, which stimulates both T and B mouse lymphocytes to proliferate¹³, also failed to induce $^{45}\text{Ca}^{2+}$ uptake in *nu/nu* spleen cells (Table 3) but did so in BALB/c spleen cells (Table 2). LPS and pokeweed mitogen failed to cause an increase in $^{45}\text{Ca}^{2+}$ uptake in *nu/nu* spleen cells even when incubated with the cells and isotopes for 60 min, instead of the standard 15 min.

Con A-induced Ca^{2+} uptake is transient

When cells were incubated at 37 °C with a mitogenic concentration of con A, or with MEM, in the presence of $^{45}\text{Ca}^{2+}$, and then sampled at various times thereafter, a con A-induced increment in the uptake of the isotope was apparent at 45 s and maximal at 1 min (Fig. 2a). Although $^{45}\text{Ca}^{2+}$ continued to be taken up by both treated and untreated cells beyond this time the difference between the two remained unchanged for a further 4 h (data not shown). This was observed in 5 out of 5 experiments, done in various media, in the presence and absence of added foetal calf serum (10%). These results suggested that the con A-induced $^{45}\text{Ca}^{2+}$ uptake was of brief duration, and this interpretation was confirmed by experiments in which the $^{45}\text{Ca}^{2+}$ was added to the cells 5 min after the con A. These showed very little difference in $^{45}\text{Ca}^{2+}$ uptake compared with the control cells (Fig. 2b). In contrast, equal amounts of $^{45}\text{Ca}^{2+}$, added as a pulse, were taken up by red cells at any time following treatment with the ionophore A23187 (data not shown), indicating that this pathway remains open.

Influence of cyclic nucleotides

Many lymphocyte responses induced by antigens or lectins can be inhibited or enhanced by manipulations which increase intracellular cyclic AMP or cyclic GMP levels respectively (reviewed in ref. 22). Whitney and Sutherland found that dibutyl cyclic AMP $\pm$ theophylline inhibited $^{45}\text{Ca}^{2+}$ uptake in human blood lymphocytes treated with PHA⁷. As can be seen in Fig. 3, treatment with dibutyl cyclic AMP (10^{-2}M – 10^{-4}M) caused a graded reduction (up to 89%) in the $^{45}\text{Ca}^{2+}$ uptake induced by mitogenic doses of con A added 30 min later. This inhibition was similar when con A was used at 0.3, 0.5, 1.0, 3.0 and 5.0 $\mu\text{g ml}^{-1}$ but the same concentrations of dibutyl cyclic AMP did not inhibit (and even enhanced) the response at con A concentrations $\geq 10 \mu\text{g ml}^{-1}$ (data not shown). Dibutyl cyclic GMP (10^{-3}M) enhanced the con A-induced $^{45}\text{Ca}^{2+}$ uptake; when both reagents were added together (10^{-4} or 10^{-3}M of each) the inhibitory effect of

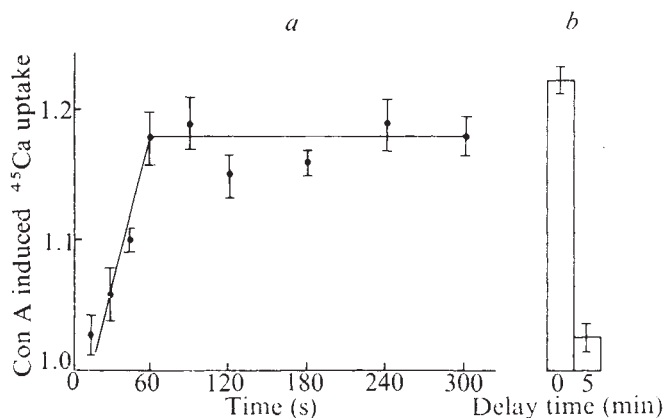


Fig. 2 a, Early time course of con A-induced $^{45}\text{Ca}^{2+}$ uptake in BALB/c spleen cells. Cells were incubated with 3 $\mu\text{g ml}^{-1}$ of con A in MEM at 37 °C and sampled at times indicated. Uncorrected induced $^{45}\text{Ca}^{2+}$ uptakes were determined as in Table 1 and are expressed as means $\pm$ s.d. of triplicates. b, Results of two experiments where $^{45}\text{Ca}^{2+}$ was added, either at time 0, or 5 min after adding con A. In each case the time of incubation with $^{45}\text{Ca}^{2+}$ was 15 min. The results are expressed as means $\pm$ s.d. of the means of the triplicates.

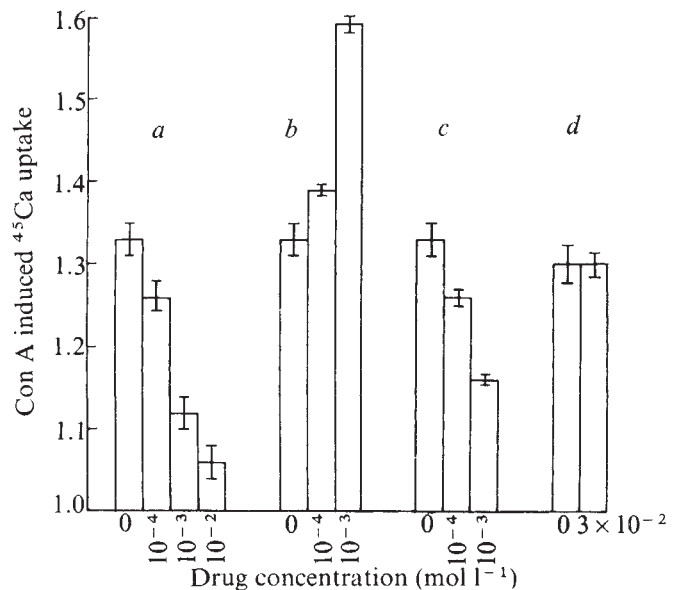


Fig. 3 Effect of a, dibutyl cyclic AMP (Sigma); b, dibutyl cyclic GMP (Sigma); c, dibutyl cyclic AMP + dibutyl cyclic GMP and d, sodium azide on uncorrected con A-induced $^{45}\text{Ca}^{2+}$ uptake. Cells were pretreated with drug or MEM at 30×10^6 cells ml^{-1} for 30 min at 37 °C before exposure to isotopes and 3 $\mu\text{g ml}^{-1}$ of con A for 15 min at 37 °C. The results of four separate experiments are expressed as means $\pm$ s.d. m. of each triplicate. In the absence of con A, these drugs did not significantly influence $^{45}\text{Ca}^{2+}$ uptake.

dibutyl cyclic AMP was overriding (Fig. 3). Neither cyclic nucleotide had a significant influence on the $^{45}\text{Ca}^{2+}$ uptake seen in cells incubated in MEM alone or with A23187. Cholera toxin ($1.2 \times 10^{-9}\text{M}$), a potent activator of adenylyl cyclase and theophylline (10^{-4}M), a phosphodiesterase inhibitor, both of which would be expected to increase intracellular cyclic AMP, also inhibited con A-induced $^{45}\text{Ca}^{2+}$ uptake, whereas 5'AMP (10^{-3} – 10^{-5}M) or butyric acid (10^{-3} – 10^{-5}M) were without effect (data not shown). Sodium azide ($3 \times 10^{-2}\text{M}$), a respiratory inhibitor, had no influence on the uptake of $^{45}\text{Ca}^{2+}$ induced by con A added 30 min later (Fig. 3).

Are gated Ca^{2+} relevant for mitogenesis?

Our studies, which follow the pioneering work of Allwood *et al.*⁶, and of Whitney and Sutherland^{7,8}, show that con A induces increased $^{45}\text{Ca}^{2+}$ uptake in mouse spleen cells, which is measurable by 45 s and complete by 1 min. Although soluble con A binds to all mouse spleen cells, only T lymphocytes are induced to divide and differentiate into lymphoblasts¹³. We have found that spleen cell populations depleted of T cells by treatment with anti- θ antiserum and complement, or derived from T-cell deficient *nu/nu* mice, do not show increments in $^{45}\text{Ca}^{2+}$ uptake on addition of con A. This proves that the increased uptake of $^{45}\text{Ca}^{2+}$ caused by con A is T-cell dependent, but does not identify the T cells unambiguously as being the cells displaying increased $^{45}\text{Ca}^{2+}$ uptake, though this seems most probable. It is unlikely that T cells are required to enable other cell types to take up $^{45}\text{Ca}^{2+}$, since the effect is (1) extremely rapid compared with T-cell secretory responses²³, (2) short and (3) not inhibited by high concentrations of sodium azide which would be expected to inhibit secretion by T cells.

It is interesting that mitogenic concentrations of bacterial LPS or pokeweed mitogen, both known to stimulate mouse B cell proliferation¹³, did not induce measurable increments in $^{45}\text{Ca}^{2+}$ uptake in spleen cells from *nu/nu* mice, even though the LPS proliferative response has been shown to depend on extracellular Ca^{2+} (ref. 5). In normal spleen cell suspensions, pokeweed mitogen did induce increased $^{45}\text{Ca}^{2+}$ uptake, probably in T lymphocytes. This may reflect a difference in the mechanism of activation in T and B cells.

It is difficult to be certain that the con A-induced $^{45}\text{Ca}^{2+}$ uptake truly reflects Ca^{2+} accumulation within the cell cytosol, and not increased adsorption at the cell surface. But the observations that the increased uptake is restricted to T cells (Table 3), that the effect is not seen if the cells are preincubated with con A for 5 min before the $^{45}\text{Ca}^{2+}$ is added (Fig. 2b) and that it can be modulated by cyclic nucleotides (Fig. 3), all suggest that it is membrane transport, and not surface adsorption that is involved. Since, in general, extracellular Ca^{2+} concentrations are high ($\geq 10^{-3}$ M) compared with the concentration in the cytosol (10^{-5} – 10^{-8} M)²⁴, the simplest interpretation of the increased $^{45}\text{Ca}^{2+}$ uptake caused by con A is that it is related to the opening of ' Ca^{2+} channels' in the plasma membrane, which enables $^{45}\text{Ca}^{2+}$ to enter the cell down a steep concentration gradient. One can calculate that the amounts of Ca^{2+} taken up by T cells stimulated by con A ($\sim 0.23 \times 10^{-9}$ M per 10^6 cells) would be large enough to raise the intracellular Ca^{2+} by 1.9 mM if all of the cell water ($\sim 122 \mu\text{M}^3$)²⁵ were available to the incoming Ca^{2+} . The rapid onset, brief duration and the resistance to inhibition by sodium azide of the induced $^{45}\text{Ca}^{2+}$ uptake are consistent with this notion of gated Ca^{2+} channels.

It is interesting that rapid opening and closing of gated Ca^{2+} channels has been proposed to explain the phenomena of histamine secretion and desensitisation in rat peritoneal mast cells induced by the binding of antigen to IgE receptors in the presence and absence of Ca^{2+} respectively^{26–28}. On the other hand, in their studies on PHA-induced $^{45}\text{Ca}^{2+}$ uptake in human blood lymphocytes, Whitney and Sutherland found no evidence of gate closure: accelerated uptake of $^{45}\text{Ca}^{2+}$ into PHA-treated cells continued for 24 h^{7,8}. Whether this important discrepancy reflects differences in species, lymphocyte populations or methodology remains to be determined. We have found that con A, PHA and pokeweed mitogen behave similarly in this respect on mouse spleen cells.

The relationship between the lectin-induced $^{45}\text{Ca}^{2+}$ uptake and DNA synthesis is far from clear. The findings that both responses occur only in T cells, show similar dose responses, and are inhibited by dibutyryl cyclic AMP and enhanced by dibutyryl cyclic GMP all suggest that they may be related. The following observations are also compatible with the idea that intracellular Ca^{2+} is a second messenger, which acts early in the sequence of events which lead to T lymphocyte proliferation and differentiation: (1) increased $^{45}\text{Ca}^{2+}$ uptake is the earliest reported response and is not inhibited by ATP depletion caused by sodium azide; (2) all the early responses so far tested (enhanced turnover of phosphatidyl inositol⁹ and increased amino acid transport²⁹) and the late responses (proliferation and differentiation)^{3–5} induced by con A or PHA have been found to be Ca^{2+} dependent; (3) pig⁹ and human¹⁰ lymphocytes can be induced to synthesise DNA and to differentiate into lymphoblasts by application of the Ca^{2+} ionophore A23187. On the other hand, the transient uptake of Ca^{2+} during the first

minute after the binding of the lectin cannot be a sufficient or irreversible signal leading to DNA synthesis, since removal of con A from the cell surface (by addition of α -methyl mannoside) at any time during the first 18 h aborts the proliferative response³⁰. Since the removal of Ca^{2+} by addition of EGTA during the first 12–36 h also prevents PHA-induced DNA synthesis in human⁴ and mouse⁵ lymphocytes, it is clear that the requirement for extracellular Ca^{2+} is also a prolonged one.

In addition to its possible relevance to lymphocyte proliferation, the ability to measure lectin-induced $^{45}\text{Ca}^{2+}$ uptake simply and reproducibly in mouse T cells opens the way to studying the properties of these putative 'gated channels' for Ca^{2+} . Such channels could be opened directly by con A binding to membrane glycoproteins which function as gating molecules, or by some less direct mechanism. The finding that dibutyryl cyclic GMP enhances, while dibutyryl cyclic AMP inhibits con A-induced $^{45}\text{Ca}^{2+}$ uptake suggests that the operation of the gating event may be regulated by these cyclic nucleotides³¹.

We thank Dr E. Simpson for supplying BALB/c and *nu/nu* mice, Dr M. Greaves for measuring the DNA synthesis in the experiment shown in Fig. 1, and Dr G. Janossy for supplying some of the mitogens. M.H.F. is supported by a Visiting Scientist Award from the Canadian MRC.

No reprints of this paper will be available.

Received March 10; accepted April 11, 1975.

- 1 Heilbrunn, L. V., and Wiercinski, F. J., *J. cell. comp. Physiol.*, **29**, 15–28 (1947).
- 2 Rubin, R. P., *Pharmac. Rev.*, **22**, 389–428 (1970).
- 3 Alford, R. H., *J. Immun.*, **104**, 698–703 (1970).
- 4 Whitney, R. B., and Sutherland, R. M., *J. cell. Physiol.*, **80**, 329–337 (1972).
- 5 Diamantstein, T., and Ulmer, A., *Immunology*, **28**, 121–125 (1975).
- 6 Allwood, G., Asherson, G. L., Davey, M. J., and Goodford, P. J., *Immunology*, **21**, 509–516 (1971).
- 7 Whitney, R. B., and Sutherland, R. M., *Cell. Immun.*, **5**, 137–147 (1972).
- 8 Whitney, R. B., and Sutherland, R. M., *J. cell. Physiol.*, **82**, 9–19 (1973).
- 9 Maino, V. C., Green, N. M., and Crumpton, M. J., *Nature*, **251**, 324–327 (1974).
- 10 Luckasen, J. R., White, J. G., and Kersey, J. H., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 5088–5090 (1974).
- 11 Boyle, W., *Transplantation*, **6**, 761–764 (1968).
- 12 Paganelli, C. V., and Solomon, A. K., *J. gen. Physiol.*, **41**, 259–277 (1974).
- 13 Greaves, M. F., and Janossy, G., *Transplant. Rev.*, **11**, 87–130, (1972).
- 14 Borjeson, J., Reisfeld, R., Chessin, L. N., Welsh, D., and Douglas, S. D., *J. exp. Med.*, **124**, 859–872 (1966).
- 15 Maino, V. C., Hayman, M. J., and Crumpton, M. J., *Biochem. J.*, **146**, 247–252 (1975).
- 16 Reed, P. W. and Lardy, H. A., in *The Role of Membranes in Metabolic Regulation* (edit. by Mehlman, M. A., and Hanson, R. W.), 111 (Academic, New York, 1972).
- 17 Harris, A. W., Bankhurst, A., Masons, S., and Warner, N. L., *J. Immun.*, **110**, 431–438 (1973).
- 18 Boylston, A., and Mowbray, J., *Immunology*, **27**, 855–861 (1974).
- 19 Greaves, M. F., Bauminger, S., and Janossy, G., *Clin. exp. Immun.*, **10**, 537–554 (1972).
- 20 Raff, M. C., *Nature*, **224**, 378–379 (1969).
- 21 Raff, M. C., *Nature*, **246**, 350–351 (1973).
- 22 Braun, W., Lichenstein, L. M., and Parker, C. W. (eds) *Cyclic AMP, Cell Growth and the Immune Response* (Springer, New York, 1974).
- 23 Bloom, B. R., *Adv. Immun.*, **13**, 102–185 (1971).
- 24 Rasmussen, H., *Science*, **170**, 404–412 (1970).
- 25 Andreasen, P. A., Schaumburg, B. P., Osterlind, K., Vinten, J., Gammeltoft, S., and Gliemann, J., *Analyt. Biochem.*, **59**, 610–616 (1974).
- 26 Foreman, J. C., Mongar, J. L., and Gomperts, B. D., *Nature*, **245**, 249–251 (1973).
- 27 Foreman, J. C., and Garland, L. G., *J. Physiol., Lond.*, **239**, 381–391 (1974).
- 28 Foreman, J. C., and Gomperts, B. D., *Int. Archs Allergy appl. Immun.* (in the press).
- 29 Whitney, R. B., and Sutherland, R. M., *Biochim. biophys. Acta*, **298**, 790–797 (1973).
- 30 Novogrodsky, A. and Katchalski, E., *Biochim. biophys. Acta*, **228**, 579–783 (1971).
- 31 Foreman, J. C., Mongar, J. L., Gomperts, B. D., and Garland, L. G., *Biochem. Pharmac.*, **24**, 538–540 (1975).

letters to nature

Spectra of high-frequency radio emission from Jupiter

BETWEEN May 1972 and October 1974, observations were made from the University of Otago's field site at Swampy Summit near Dunedin, of Jupiter's high-frequency radio emissions. The frequency range of 23–25 MHz was reduced to a 2 MHz video

signal, recorded on videotape, and analysed with a high resolution spectrum analyser to produce the dynamic spectrum of the millisecond radio bursts. The spectrum analyser was constructed from a modified video tape recorder, using the time expansion principle developed independently by Ellis¹. The frequency and time resolution were 10 KHz and 500 μs , respectively, and the spectrum was recorded on 35 mm film at the rate of 20, 40 or 100 ms per half frame.

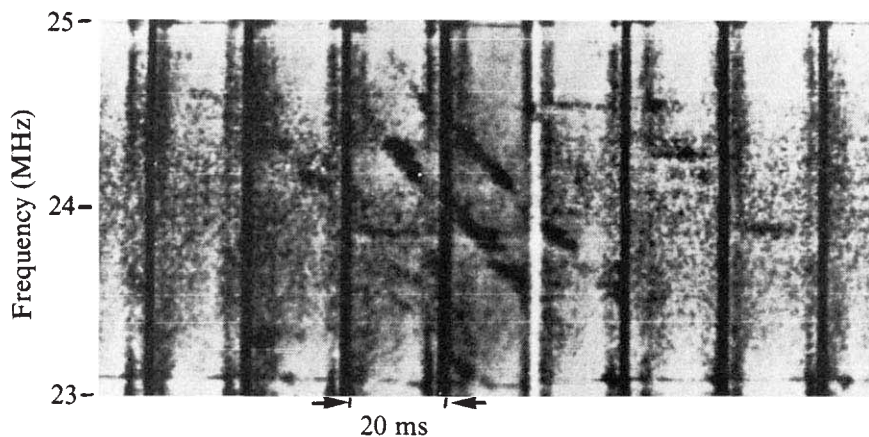
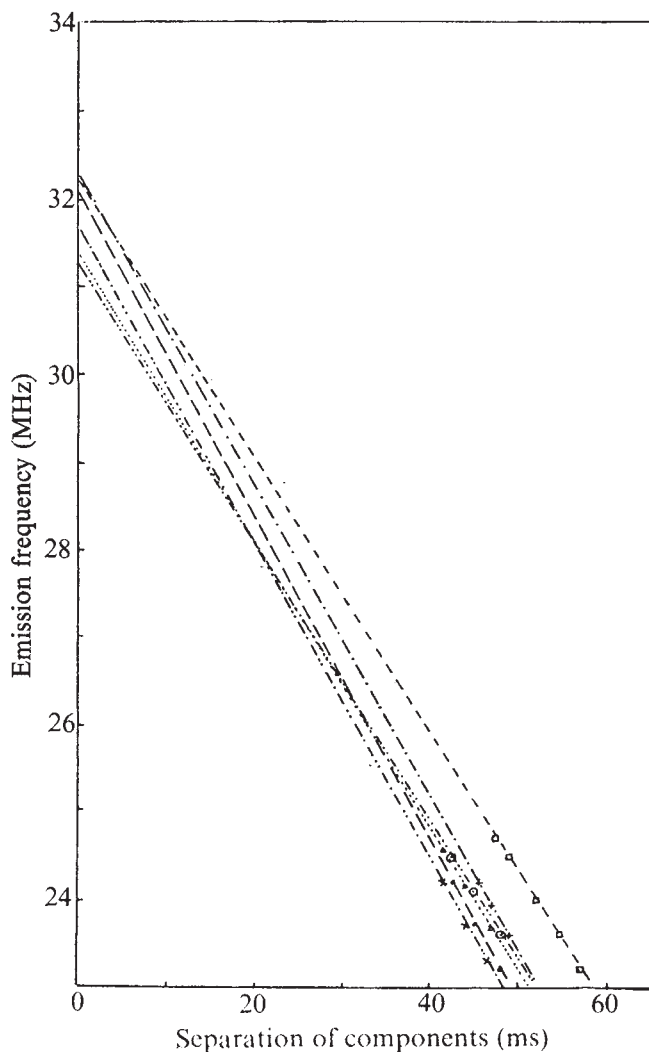


Fig. 1 The dynamic spectrum of Jupiter radio bursts: a closely-spaced doublet emission.

During the early source event of August 3, 1973, more than 75 min of videotape records were taken of the Jupiter radio bursts, producing over 1 km of film record showing more than 600 cases of millisecond burst spectra. Most of the emissions occurred in three burst groups lasting a total of less than 5 min, with the strongest burst group lasting for about 30 s.

A typical millisecond burst comprises a falling tone with a bandwidth of 20–150 KHz, and a frequency-time slope of -25 to -30 MHz s^{-1} . These values are in contrast with those

Fig. 2 Separation of components in several widely-spaced millisecond bursts, as a function of frequency. The separations extrapolate back to zero in the region of 32 MHz.



of -10 to -15 MHz s^{-1} reported by Ellis², for observations at 15 to 17 MHz; they are, however, consistent with his predictions for cyclotron radiation from bunches of electrons moving along the spatially varying magnetic field of Jupiter.

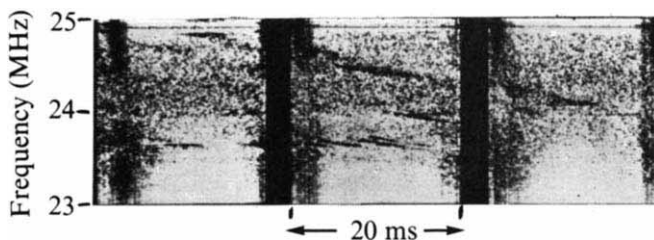
Multiple s-bursts which were proposed by some early observers but not confirmed, were common during periods of high emission rate. These occurred in pairs, and occasionally in triplets, with the component bursts each time having about the same frequency range, amplitude and slope. Separations between the components generally fell into the range 45–55 ms (widely spaced bursts), or 15–25 ms (closely spaced bursts), (Fig. 1).

A curious feature of the widely-spaced bursts is that the frequency-time traces of bursts are not quite parallel, but show consistent divergence, such that if the traces are extrapolated back in time they coincide at around 32 MHz. This extrapolation for several burst pairs and two triplets is shown in Fig. 2, where measurements of the component separation are plotted as a function of frequency over the observed range. The triplets are of particular interest, in that not only do the components extrapolate back to a common origin, but the middle component is also midway between the outer two at all frequencies (within the accuracy of measurement). It is difficult to determine whether the closely-spaced multiple bursts also show this divergence, as most of those bursts were too short for accurate measurement.

On a millisecond time scale, a considerable amount of fine structure was observed in the emission spectra (Fig. 3). Several discrete millisecond bursts were found to comprise shorter emissions with a time separation of 2–5 ms, and a frequency separation of 100–200 KHz. Other seemingly narrow-band bursts were actually made up of two or more very narrow band emissions (~ 20 KHz), with frequency separations down to 50 KHz. These emissions may be related to the fast lane bursts reported by Ellis, or could be a form of very closely spaced multiple bursts.

The properties of the multiple bursts fit Ellis's cyclotron model if we suppose that the components are emissions from closely spaced electron bunches moving in tandem along a

Fig. 3 High resolution dynamic spectrum of Jupiter s-bursts showing both a burst with overlapping structure and a burst with fine structure components with a frequency separation of ~ 70 kHz.



field line at about the same velocity as one another. The divergence with descending frequency of the temporal separation of the burst components and thus of the spatial separation of the electron bunches with distance up the field line, suggest that the bunches are formed at levels where the local cyclotron frequency is ~ 32 MHz. The ordered spacing of the triplet components suggest that there is an ordered process of bunch formation, such as the breaking up of electron streams because of plasma instability. Only two strong triplets of adequate frequency range were, however, observed in this event. Clearly, more observations, preferably extending up to higher frequencies, would be helpful.

This work was supported by a grant from the New Zealand University Grants Council Research Committee.

M. J. GROTH
R. L. DOWDEN

Department of Physics,
University of Otago,
Dunedin, New Zealand

Received March 10; accepted April 9, 1975.

¹ Ellis, G. R. A., *Aust. J. Phys.*, **26**, 253 (1973).
² Ellis, G. R. A., *Nature*, **235**, 415 (1975).

Low frequency variability of BL Lac

THE unusual object BL Lacertae has attracted attention because of its large and erratic variability at optical, millimetre and centimetre wavelengths¹⁻³. The general form of the variations at short radio wavelengths is compatible³⁻⁵ with the models of Van der Laan⁶ and Ozernoy and Sazonov⁷. We report here observations of the variability of BL Lac at relatively long radio wavelengths which are in serious conflict with these models.

The observations were made at Jodrell Bank at $\lambda 74$ cm (408 MHz) and $\lambda 31$ cm (962 or 966 MHz) between July 1969

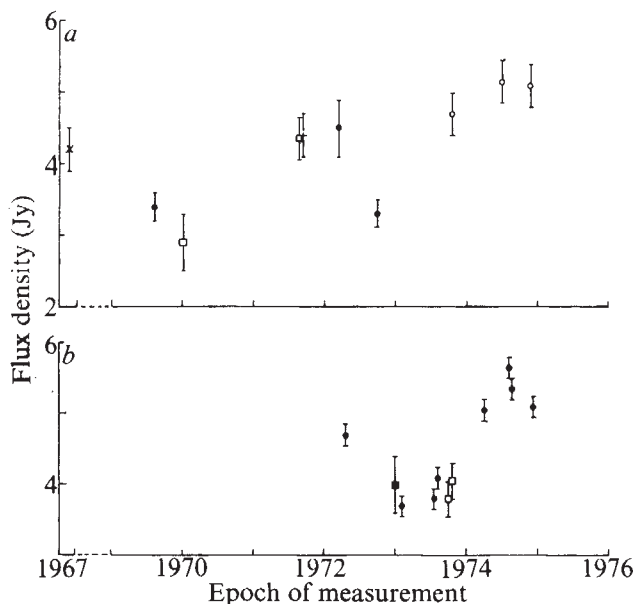


Fig. 1 The flux density of BL Lac at: *a*, $\lambda 74$ and *b*, $\lambda 31$ cm. ■, Measurements with the Mk IA single dish radiotelescope; ●, measurements with the Mk IA-II 425-m interferometer; □, measurements with the Jodrell-MkIII 23.8-km radio link interferometer; ○, measurements with the Jodrell-Defford 127.3-km radio link interferometer. + and ×, Measurements at $\lambda 74$ cm made at Bologna and Cambridge, respectively. The significance of the error bars is discussed in the text.

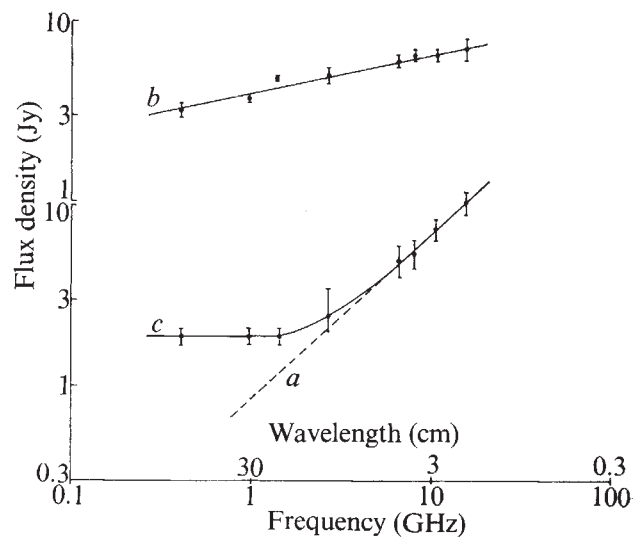


Fig. 2 The spectra of the quiescent and active components of BL Lac. *a*, Activity predicted at long wavelengths by the expanding cloud models; *b*, quiescent component; *c*, active component.

and December 1974. Extensive observations with the Jodrell-Mark III radio link interferometer show that BL Lac is completely unresolved at both wavelengths, with upper limits to its angular diameter of 2.0 arc s at $\lambda 74$ cm and 1.0 arc s at $\lambda 31$ cm. More limited observations with the Jodrell-Defford interferometer suggest that the angular diameter at $\lambda 74$ cm is in fact smaller than 0.4 arc s. Because of this compact size, fringe amplitude measurements made with both short and long baseline interferometers at Jodrell Bank can be taken to provide measurements of the total flux density of BL Lac.

The measurements of flux density are shown as a function of epoch of observation in Fig. 1. The data obtained at $\lambda 74$ cm are given on the flux density scale of Kellermann *et al.*⁸ (KPW), with the assigned errors containing an allowance for the systematic uncertainties produced in converting the measurements made with different instruments (and often different calibration sources) to this common scale of flux density. Observations of non-variable sources with the same instruments suggest that the error estimates correspond to confidence limits at about the 90% level. The figure includes observations made at $\lambda 74$ cm by Willson⁹, and Fanti (personal communication). The data at $\lambda 31$ cm were obtained primarily using the Mark IA-II interferometer, and have been calibrated on the nominal KPW scale at this wavelength by assuming the calibration source 3C295 to have a flux density of 29.8 Jy. The errors assigned to these measurements reflect only the amplitude repeatability of 3C295 and other calibration sources. For comparison with measurements made with other instruments and calibration sources, the errors shown on the Mark IA-II interferometer measurements at $\lambda 31$ cm should be increased to ± 0.25 Jy. The errors assigned to the $\lambda 31$ cm measurements with the Jodrell-Mark III interferometer and the Mark IA single dish are the full formal errors on the KPW scale.

The data in Fig. 1 show that significant variations of about 2 Jy have occurred in the flux density of BL Lac at both $\lambda 74$ and $\lambda 31$ cm. Because of the uneven and often lengthy interval between the long wavelength flux density measurements we are unable to make a detailed comparison with individual events at short wavelengths. It is, however, possible to compare the general levels of activity using the procedure adopted by Ekers *et al.*⁵. Systematic monitoring at short wavelengths suggests that the activity of BL Lac can be well represented by a succession of discrete outbursts which are superimposed on some relatively stable 'quiescent' level. The physical significance of this base level is unclear; it may represent a steady non-varying component of the source, or it may merely be a

complex level produced by the superposition of a number of decayed or small events. Whatever the cause, the apparent stability of the level does provide a useful reference, above which the activity can be defined.

We have used data from other variability studies^{3-5,10-14}, together with our own data, to assign values to the quiescent and active components of BL Lac between $\lambda 1.9$ and $\lambda 74$ cm. At each wavelength we have averaged the deepest minima in the intensity to define the base level of the quiescent component, and several of the highest peaks to obtain the general level of activity above this value. Because of the limited amount of data at $\lambda 74$ and $\lambda 31$ cm, the values we have assigned to the active component may well be an underestimate of the true activity at these wavelengths. The variability studies have, in general, differing time spans, so that it is necessary to assume that the level of activity at each wavelength did not change markedly between 1968 and 1974. The almost continuous runs of data by Medd *et al.*¹⁰ and Andrew⁴ suggest that this was the case at both $\lambda 2.8$ and $\lambda 4.5$ cm from 1968 until near the end of the summer of 1972. The variability seemed to cease for several months at this time, but we assume that this was only a temporary lapse, and that the activity at short wavelengths has since returned to its more usual level.

The spectra of the quiescent and active components of BL Lac are shown in Fig. 2. The quiescent component has an inverted spectrum with a frequency spectral index of approximately $+0.2$ (defined in the sense that $S \propto f^\alpha$). The variable component is flatter than this at low frequencies, but considerably steeper at high frequencies. As suggested by Ekers *et al.*⁵, this marked difference in spectral characteristics supports the view that the quiescent spectral component may be associated with a discrete non-variable component of the source.

The activity of BL Lac at short wavelengths is in good general agreement³⁻⁵ with the predictions of the expanding cloud model^{6,15,16} and the application⁷ of this model to ejected components. Although these models may not explain the detailed profiles of individual outbursts entirely satisfactorily⁵, they do predict well the observed time delay between events at different wavelengths, and the inverted power law spectrum of the activity. The activity predicted by the models at long wavelengths is much less than that actually observed (Fig. 2a).

Not only do the conventional models for variability fail to account for the amount of activity at long wavelengths, but there is also a suggestion in the present $\lambda 31$ cm data that the time scale of the activity may be somewhat different at these wavelengths. At short wavelengths BL Lac has an average of about 3 or 4 prominent outbursts each year. The activity at $\lambda 31$ cm seems to have a considerably longer time scale of about 3 yr. Though it is true that our observations would not be particularly sensitive to variations on a time scale of a few months, there is little indication in the data of extensive short term activity of the type observed at short wavelengths. For instance, during a 30-d period in July and August 1973 we made almost daily observations of the flux density of BL Lac at $\lambda 31$ cm. The measurements show only a gradual rise in intensity of about 0.3 Jy, with no evidence of short duration activity. At $\lambda 74$ cm the observations are so infrequent that we can say little about the time scale of the variations. It is interesting to note, however, that the variations are superficially similar at both $\lambda 31$ and $\lambda 74$ cm, and can be fitted by a smooth curve which is in phase at the two wavelengths.

Hunstead¹⁷ has reported variations in the $\lambda 74$ cm flux density of the quasars 3C454.3 and CTA102, and the radio galaxies 1504-16.7 and 1524-13. The time scale of these variations is very similar to that of the variability reported here for BL Lac. Observations of the two quasars by Medd *et al.*¹⁰ suggest that the activity at short wavelengths is apparently unconnected with the long wavelength variations. This is also true for BL Lac, and it is tempting to speculate that the same type of mechanism may be responsible for the low frequency activity in all five sources. The nature of this mechanism is as yet unknown.

We are grateful to colleagues for assistance with the observations, and thank R. G. Strom and P. N. Wilkinson for providing the early epoch measurements at $\lambda 74$ cm. We thank R. Fanti and the Bologna group for permission to include their unpublished measurement A.M.T., R.W.P. and R.J.D. acknowledge financial support from the Science Research Council.

D. STANNARD
A. M. TREVERTON
R. W. PORCAS
R. J. DAVIS

University of Manchester,
Nuffield Radio Astronomy Laboratories,
Jodrell Bank, Cheshire, UK

Received March 10; accepted April 11, 1975.

- 1 MacLeod, J. M., and Andrew, B. H., *Astrophys. Lett.*, **1**, 243 (1968).
- 2 Bertaud, C., *et al.*, *Astr. Astrophys.*, **24**, 357 (1973).
- 3 MacLeod, J. M., Andrew, B. H., Medd, W. J., and Olsen, E. T., *Astrophys. Lett.*, **9**, 19 (1971).
- 4 Andrew, B. H., *Astrophys. J. Lett.*, **186**, L3 (1973).
- 5 Ekers, R. D., Weiler, K. W., and van der Hulst, J. M., *Astr. Astrophys.*, **38**, 67-73 (1975).
- 6 van der Laan, H., *Nature*, **211**, 1131 (1966).
- 7 Ozernoy, L. M., and Sazonov, V. N., *Astrophys. Space Sci.*, **3**, 395 (1969).
- 8 Kellermann, K. I., Pauliny-Toth, I. I. K., and Williams, P. J. S., *Astrophys. J.*, **157**, 1 (1969).
- 9 Willson, M. A. G., *Mon. Not. R. astr. Soc.*, **156**, 7 (1972).
- 10 Medd, W. J., Andrew, B. H., Harvey, G. A., and Locke, J. L., *Mem. R. astr. Soc.*, **77**, 109 (1972).
- 11 Dent, W. A., and Kojoian, G., *Astr. J.*, **77**, 819 (1972).
- 12 Witzel, A., and Veron, P., *Astrophys. Lett.*, **7**, 225 (1971).
- 13 Dent, W. A., Kapitzky, J. E., and Kojoian, G., *Astr. J.*, **79**, 1232 (1974).
- 14 Hackney, R. L., *et al.*, *Astrophys. Lett.*, **12**, 147 (1972).
- 15 Shklovsky, I. S., *Astr. Zh.*, **37**, 256 (1960).
- 16 Pauliny-Toth, I. I. K., and Kellermann, K. I., *Astrophys. J.*, **146**, 634 (1966).
- 17 Hunstead, R. W., *Astrophys. Lett.*, **12**, 193 (1972).

The output of the Etna volcano

ETNA shows two types of eruptive activity: persistent activity during which lava issues continuously at a low volumetric rate over a long time, and periodic eruptions during which lava issues at a high rate over a short time. The former is from vents at or near the summit, the latter most commonly from flank vents. In some flank eruptions the lava degasses from the central vent, and in others partly from the flank vents, building scoria cones there.

The historical record of activity on Etna is unrivalled in the large number of lava flows of known date and areal extent: Sartorius von Waltershausen¹ compiled a map of the lavas formed before 1839, and each eruption since then has been documented. Nevertheless, few attempts have been made to determine the volumetric output of new rock over the historic period, in spite of the importance of such data to understanding the mechanism of the volcano.

The problem of determining the output is not, however, straightforward, because although the volume of each dated, flank lava flow can be readily estimated, it is difficult to assess the contribution from persistent summit activity. We consider here the present output and attempt to relate this to the earlier history.

The years immediately before 1971 were characterised by persistent activity from the north-eastern crater. During that time we made estimates of the effusion rate on three occasions (refs 2 and 3, and G. P. L. W., unpublished) and arrived at a consistent value of around $1 \text{ m}^3 \text{ s}^{-1}$. The accumulated products of the persistent summit activity from 1932 to 1967 amount to $270 \times 10^6 \text{ m}^3$, based on a comparison of two editions of the 1:25,000 topographic map of Etna; flank activity during this period contributed a further $60 \times 10^6 \text{ m}^3$, giving a total of $330 \times 10^6 \text{ m}^3$ or $0.3 \text{ m}^3 \text{ s}^{-1}$ averaged over this period of 35 yr.

The persistent activity ceased with the commencement of an eruption during the spring of 1971 but resumed again in late

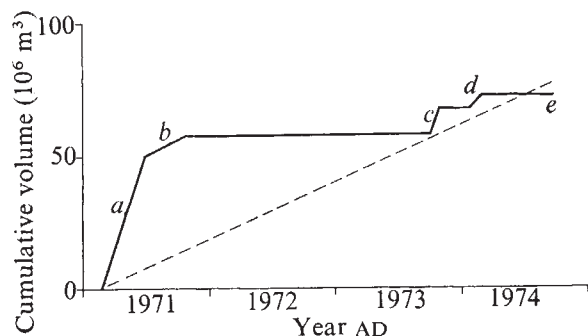


Fig. 1 Cumulative output of Etna by summit and flank activity during the period April 1971–September 1974. *a*, 1971 eruption; *b*, partial infilling of the Chasm; *c*, partial infilling of the Chasm and Bocca Nuova; *d*, January–March 1974 flank eruption; *e*, resumption of persistent activity from the North-eastern Crater. Dashed line, average output rate ($0.68 \text{ m}^3 \text{ s}^{-1}$).

1974. Between the 1971 eruption and late 1974 there were, however, several eruptive events, notably a partial infilling of the Chasm (the main crater at the summit of Etna) by lava in 1973. A small flank eruption also occurred in January–March 1974 (ref. 4). The estimated volume of lava produced during all the activity from spring 1971 to the present (Fig. 1) gives an average rate of $0.68 \text{ m}^3 \text{ s}^{-1}$. These figures compare with the higher average rate of $3.4 \text{ m}^3 \text{ s}^{-1}$ for Kilauea⁵.

Reasonably complete records of flank eruptions span the period from 1535 to the present. One of us⁶ has measured the area of each historic flank flow and has visited each locality to estimate the average thickness of the flows. The assessed volumes (Fig. 2) are, in general, smaller than volumes given in the literature, but if there is any subjective error in estimating the thickness of the flows it should be consistent.

The striking feature (Fig. 2) is that there were four periods of contrasted outputs: first, 1535–1610, characterised by a rather low effusion rate; second, 1610–1669, characterised by a high effusion rate, averaging $0.83 \text{ m}^3 \text{ s}^{-1}$; third, 1669–1763, during which there were no major flank eruptions; fourth, from the 1759 resumption of flank activity to the present, characterised by a uniform and moderate effusion rate averaging $0.17 \text{ m}^3 \text{ s}^{-1}$.

The high effusion rate between 1610 and 1669 could be correlated with the absence of summit activity during that period: flank eruptions accounted for the entire output. Following that period a large caldera formed at the summit during the 1669 flank eruption, and the contemporaneous absence of flank eruptions could have been because the steady volcanic activity at the summit was filling in the caldera. By the time of Spallanzane's visit at the end of the eighteenth century⁷, Etna again had a summit cone standing above the caldera rim.

Persistent summit activity occurred more or less continuously between 1759 and the present, and the average effusion rate of $0.17 \text{ m}^3 \text{ s}^{-1}$ resulting from the flank activity is thus only part of the total. It is impossible to arrive at an accurate figure for the contribution by persistent summit activity. In particular, there are many undated lava flows which originated high on the volcano belonging to that period. A rough calculation of the volume of Etna standing above the 1669 caldera rim, and the volume of known summit lava flows of the past 200 yr gives a total of $500 \times 10^6 \text{ m}^3$, and an average effusion rate of $0.09 \text{ m}^3 \text{ s}^{-1}$. Some pyroclastics have fallen beyond the caldera rim or have been eroded from the summit cone, but a total output of close to $0.26 \text{ m}^3 \text{ s}^{-1}$ for the period is indicated.

A volcano such as Etna is a system into which magma rises from the mantle and out of which lava is erupted at the surface. The input is generally greater than the output since some, perhaps rather more than 10% of the magma which enters the volcano congeals below the surface to form intrusions. There is general evidence that the input is continuous, and that high

level magma reservoirs exist, within which some of the input is stored between eruptions.

A model which can reconcile the varied output over the past 450 yr with a uniform input rate is one in which there are two magma reservoirs within or below Etna filling and emptying independently of one another. The smaller reservoir would have a capacity of the order of $50 \times 10^6 \text{ m}^3$ and would be partially or totally drained during normal flank eruptions such as have occurred roughly every 10 years between 1763 and the present. The large reservoir would be of an order of magnitude larger and would probably take hundreds of years to fill: the last time it emptied was in the seventeenth century. The most cogent evidence for the existence of this larger reservoir is the caldera collapse which took place at the summit of Etna during the 1669 flank eruption and terminated the period of high output (Fig. 2).

On this model, the input rate is constant at perhaps 0.3 – $0.4 \text{ m}^3 \text{ s}^{-1}$. The output during the period 1610–1669 was greater because of the release of the contents of the larger reservoir which had filled over a long period. The output during the two later periods was smaller because slow refilling of the large reservoir then absorbed part of the input.

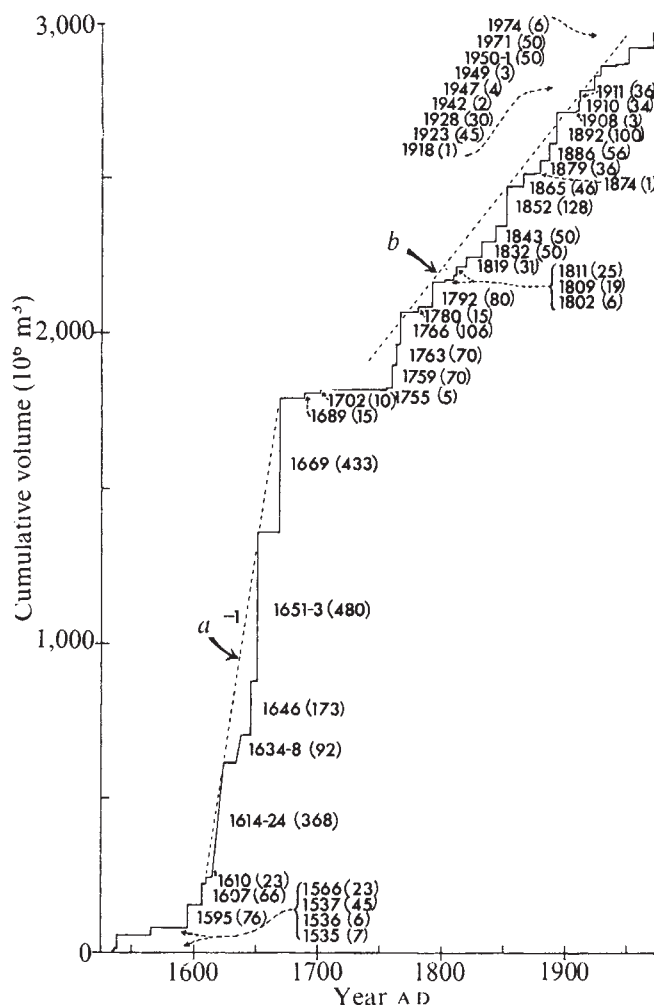


Fig. 2 Cumulative output of Etna by flank activity since 1535. Some of the eruptions listed in ref. 9 are omitted (1540, 1550, 1578–80—small eruptions, no volumetric data available; 1883—no lava flow produced; 1682, 1747, 1869—summit eruptions; 1764–45—confusion exists regarding the source, but very probably persistent activity; 1603—dubious eruption), but probably do not change the picture significantly. *a*, Average output rate: $0.83 \text{ m}^3 \text{ s}^{-1}$; *b*, average output rate: $0.17 \text{ m}^3 \text{ s}^{-1}$. Numbers in brackets following dates give the volume of output (10^6 m^3) for those dates.

A second model which can reconcile a varied output with a uniform input requires only one storage reservoir, and the nature of the eruptive activity depends on the state and, particularly, the height of the volcano. Persistent activity is a steady leakage which acts as a form of safety valve. Perhaps it can release the entire input, although it normally releases only a part and flank eruptions release the remainder. Persistent summit activity steadily increases the height of the volcano, and because the hydrostatic pressure in magma at the summit decreases as its height increases⁸ this results in a reduced leakage rate. A greater proportion of the input is stored, and the resulting inflation of the volcano then increases the likelihood of major flank outbreaks, as in the seventeenth century. The major channelways so opened divert summit activity to the flanks. The loss of a large volume of magma from the storage reservoir results in caldera collapse, thus terminating a cycle. One possible objection to this model is that the height of Etna has not increased significantly in the past century (1864—3,313 m; 1900—3,274 m; 1973—3,316 m). Also, a gradual decrease in leakage rate could be expected to lead to a gradual increase in the magnitude of flank eruptions, rather than an abrupt increase as occurred in the seventeenth century.

Both of these models envisage a uniform rate of input, but another possibility is that the input itself varies with time and was particularly high during the period of high output in the seventeenth century. The eruptions of this period include two (those of 1614–24 and 1651–53) which produced pahoehoe instead of the more normal aa flows of Etna, and these flows probably had an unusually low viscosity. If the low viscosity resulted from a difference in chemical composition then that, and the increased output, could well correlate with changed conditions in the mantle. The large volume of lava which emerged in the 1669 eruption, however, can be related to the unusually low level of the 1669 vents⁹.

There is at present no way to choose between these three alternative explanations for the variation in output. Whichever applies, we see signs of a cyclic pattern, each cycle lasting for some hundreds of years and terminated by caldera collapse. There are traces of two calderas (the Valle del Leone and Cratere Ellittico) which existed earlier than that of 1669 (the Cratere del Piano), and the Valle del Bove may be another. There are also lava flows of large volumes, which are older than the seventeenth century.

The discrepancy between the low output ($0.26 \text{ m}^3 \text{ s}^{-1}$) of the past 200 yr and the higher output of the most recent period ($0.68 \text{ m}^3 \text{ s}^{-1}$) could be attributable to several factors. One possibility is that the latter indicates the onset of another highly voluminous effusive period such as that of the seventeenth century, although there is no other supporting evidence for this.

The base level of Etna varies from below sea level to more than 1,000 m OD. The volume of Etna standing above a horizontal plane 500 m OD is 515 km^3 . To this should be added 12 km^3 : the volume deficit of the Valle del Bove. The total output since 1535 is of the order of 3.6 km^3 , or an average of $0.26 \text{ m}^3 \text{ s}^{-1}$. At this rate Etna could have been constructed in 65,000 yr.

Understanding the mechanism of Etna depends on the availability of good quantitative data. This study demonstrates the importance of making more critical and more frequent measurements of the output.

G. WADGE
G. P. L. WALKER

Department of Geology,
Imperial College of Science and Technology,
London SW7 2BP, UK

J. E. GUEST

University of London Observatory,
Mill Hill Park,
London NW7 2QS, UK

Received March 18; accepted April 15, 1975.

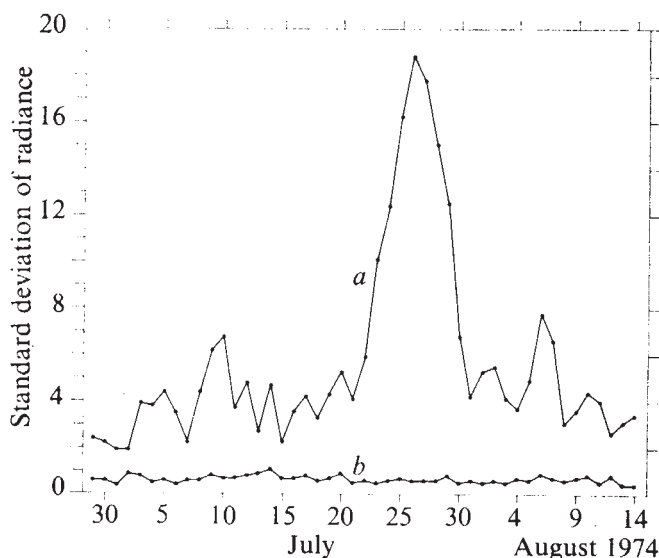
- ¹ Sartorius von Waltershausen, W., *Atlas der Aetna* (Engelman, Leipzig, 1848).
- ² Walker, G. P. L., *Nature*, **213**, 484–485 (1967).
- ³ Guest, J. E., *Phil. Trans. R. Soc.*, **A274**, 63–78 (1973).
- ⁴ Guest, J. E., *et al.*, *Nature*, **250**, 385–387 (1974).
- ⁵ Swanson, D. E., *Science*, **175**, 169–170 (1972).
- ⁶ Wadge, G., thesis, Univ. London (1974).
- ⁷ Spallanzani, A. L., *Travels in the two Sicilies*, 1 (Robinson, London, 1798).
- ⁸ Walker, G. P. L., *Misc. Pap., geol. Soc. Lond.*, **3**, 23–41 (1974).
- ⁹ Imbo, G., *Catalogue of active volcanoes of the world. Part XVIII, Italy*. (International Association of Volcanology, Rome, 1965).

Large sudden warming in the Southern Hemisphere

LARGE and rapid temperature changes, 'sudden warmings', can occur in the stratosphere during winter¹. They are hemispheric-scale phenomena, and, until recently, were supposed to be much weaker in the Southern Hemisphere winter than in the Northern Hemisphere winter. But during July 1974 a warming occurred in the Southern Hemisphere which was more intense in the initial phase than any of the three very large warmings of the past four Northern Hemisphere winters. The relatively early date of this warming (most large warmings in the Southern Hemisphere occur after late August at the end of the winter), its failure to produce the large heating of the polar cap which normally occurs in the Northern Hemisphere after such intense initial activity, and the large associated cooling in the tropics and Northern Hemisphere are further reasons for regarding this as a significant event.

Measurements by the Selective Chopper Radiometer (SCR)^{2–5} carried by Nimbus 5, give continuous coverage of atmospheric temperature between 80°S and 80°N. Uninterrupted measurements of a layer approximately 20 km thick and centred near 150 Pa (1.5 mbar) (~45 km near the stratopause) have been made since April 1970 using this instrument or its predecessor on Nimbus 4. Both instruments operate by measuring thermal emission from atmospheric carbon dioxide in the 15- μm band in the infrared, using cells of carbon dioxide acting as filters in series with interference filters. The satellites are in Sun-synchronous polar orbits, passing over most places near local noon and midnight; the orbits are 26.8° longitude apart.

Fig. 1 Standard deviation of channel B23 radiance ($\text{mW m}^{-2} \text{sr}^{-1} (\text{cm}^{-1})^{-1}$) around latitude circles. This channel measures emission from the upper stratosphere. a, 60°S; b, Equator.



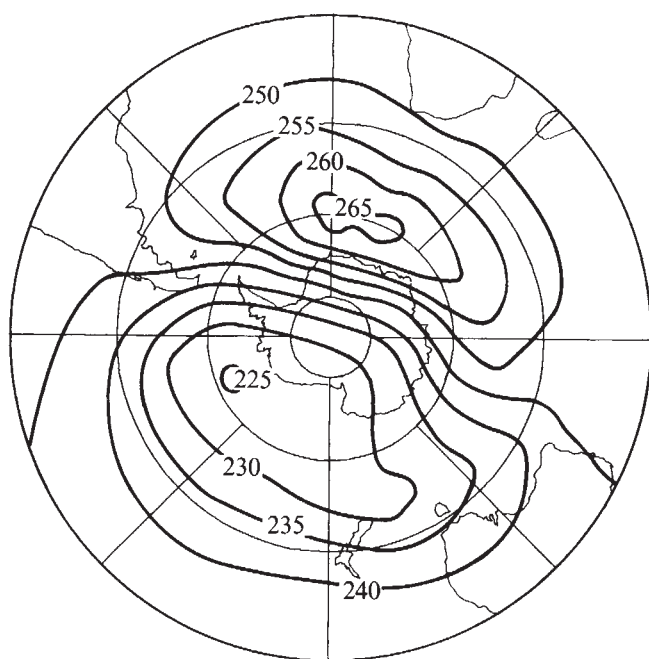


Fig. 2 Channel B23 equivalent temperature (K) on July 26, 1974, on a polar stereographic projection of the Southern Hemisphere bounded at 20°S.

A measure of the eddy activity (deviation from zonal symmetry) in the winter stratosphere is given by the temperature variation around a latitude circle at a middle or high latitude. Variations are normally largest at 60–65° latitude as in this case, and Fig. 1 gives standard deviations for channel B23 at 60°S. The weighting function of this channel has a peak near 400 Pa (~33 km); most of the measured emission originates in the 1,600–140 Pa layer (~28–46 km). The radiance unit is $\text{mW m}^{-2} \text{sr}^{-1} (\text{cm}^{-1})^{-1}$, and for points in this figure an interval of one radiance unit is equivalent to about 1 K temperature change. A large peak of 18.8 radiance units was centred on July 26, 1974; for nine days standard deviations were larger than the norm of about five units for that winter. Figure 2 gives an analysis for this day and shows that the pattern was almost entirely wavenumber one, with a hot centre at 60°S 10°E and a cold centre at 64°S 120°W. Table 1 gives the maximum standard deviations around latitude circles for the largest

Table 1 Warmings between April 1970 and February 1975 during which standard deviation of radiance around a latitude circle exceeded 12 radiance units

Date	Channel	Latitude	Standard deviation
Nimbus 4			
August 5, 1970	A	48°S	14.8
September 5, 1970	A	60°S	15.4
September 23, 1970	A	60°S	16.6
October 5, 1970	A	60°S	13.2
October 18, 1970	A	68°S	16.3
January 3, 1971	A	64°N	31.3
March 14, 1971	A	72°N	22.0
September 7, 1971	A	60°S	14.7
January 10, 1972	B	68°N	14.1
February 13, 1972	A	60°N	17.7
March 26, 1972	A	64°N	17.7
July 29, 1972	A	64°S	14.4
Nimbus 5			
January 28, 1973	B23	68°N	18.0
August 16, 1973	B23	64°S	12.7
February 25, 1974	B23	68°N	13.3
March 16, 1974	B12	64°N	13.3
July 26, 1974	B23	60°S	18.8
December 30, 1974	B23	68°N	21.2

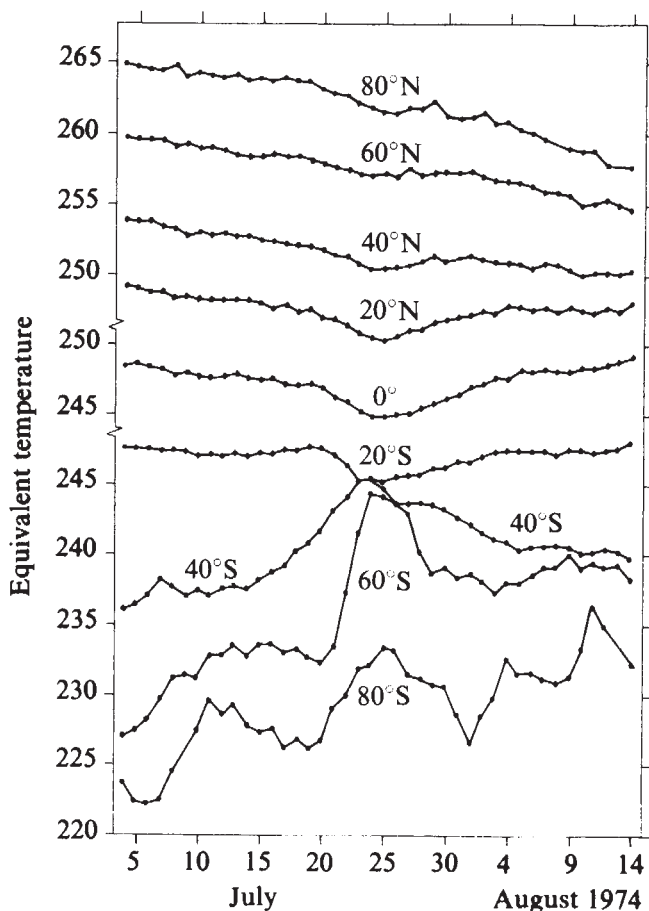
warmings observed since April 1970. Not only was the warming of July 1974 the largest Southern Hemisphere warming, but it was the earliest, and was weaker than only the two Northern Hemisphere warmings of 1971 and that of December 1974.

Large wave activity is normally accompanied by a rapid temperature rise at the pole because heat is transported poleward by the eddies at an increased rate. Figure 3 shows a temperature maximum on July 25 at 80°S, temperature having increased by 7 K during the previous 6 d. But the Northern Hemisphere warmings of January 1971, January 1973, February 1974 and December 1974 produced rises of zonal mean temperature at 80°N of about 33 K, 25 K, 35 K and 36 K respectively at this level, to give higher temperatures than at the Equator, although all except the first and last were associated with smaller eddy maximum standard deviations than that of July 1974.

Figure 3 shows that the rise of zonal mean temperature at 60°S was larger than at 80°S, although still small by Northern Hemisphere standards. A better measure of the height of these peaks of zonal mean temperature is given by the average of the rise before the peak and the fall afterwards. This was still largest at 60°S, 8.9 K compared with 7.0 K at 80°S. The maximum at these latitudes was accompanied by a minimum between 20°S and 40°N (Fig. 3), this being most marked at the Equator where temperature was 2.7 K lower on July 25 than the average of July 19 and August 1. Such tropical and summer hemisphere cooling is well known to occur with large Northern Hemisphere warmings^{7,8} and has been demonstrated for a Southern Hemisphere warming⁷, but this is the largest cooling reported (or observed by the SCRs) for a Southern Hemisphere warming.

The pattern of events differed from that which is normal in

Fig. 3 Temperature (K) equivalent to the zonal mean radiance measured by channel B23 (upper stratosphere). Values are given each day.



the Northern Hemisphere. There the maximum of zonal mean temperature is normally most pronounced at the pole and is about eight times as large as the low latitude and Southern Hemisphere cooling, which occurs simultaneously; the large eddy activity normally precedes the maximum by at least a day. Here the maximum eddy amplitude coincided with the extremes of zonal mean temperature, although the equatorial cooling was 30% of the maximum temperature rise, which occurred not at the pole but at 60°S. Similar but smaller changes in the zonal mean were shown by Fritz and Soules⁷ for a lower level in the Southern Hemisphere.

Why did the large amplitude waves during July 1974 not lead to large polar heating? Data from other levels show that the temperature anomalies tilted towards the west with increasing height and decreasing distance from the Equator. These tilts are instrumental in producing heat and momentum fluxes⁹, the relative and absolute magnitudes of which must determine the behaviour and size of the warming. But the tilts were about half as steep as has been typical in a Northern Hemisphere warming, and the wave amplitudes in the lower stratosphere were also very much smaller. Both these factors imply reduced eddy fluxes, so perhaps the pertinent question is why the equatorial cooling was so large when changes of average temperature at high latitudes were not so extreme. These changes had no significant effect on the global mean radiance at any level, so the warming merely redistributed the heat horizontally, as would result from horizontal eddy heat flux or adiabatic heating as a result of meridional circulations¹⁰.

I thank Dr J. T. Houghton and members of this department for assistance, and the SRC and the NERC for financial support. NASA provided technical support.

J. J. BARNETT

Department of Atmospheric Physics,
Clarendon Laboratory, Oxford, UK

Received April 7; accepted April 23, 1975.

- ¹ Scherhag, R., *Ber. dt. Wetterd.*, 6, 51–63 (1952).
- ² Ellis, P. J., *et al.*, *Nature*, 228, 139–143 (1970).
- ³ Ellis, P. J., *et al.*, *Proc. R. Soc. A*, 334, 149–170 (1973).
- ⁴ Barnett, J. J., *et al.*, *Nature*, 230, 47–48 (1971).
- ⁵ Barnett, J. J., *et al.*, *Q. Jl R. met. Soc.*, 98, 17–37 (1972).
- ⁶ Barnett, J. J., *et al.*, *Atmospheric Physics Memorandum*, 74.1 (Clarendon Laboratory, Oxford, 1974).
- ⁷ Fritz, S., and Soules, S. D., *Mon. Weath. Rev.*, 100, 582–589 (1972).
- ⁸ Barnett, J. J., *Q. Jl R. met. Soc.*, 100, 505–530 (1974).
- ⁹ Muench, H. S., *J. atmos. Sci.*, 22, 349–360 (1965).
- ¹⁰ Matsuno, T., *J. atmos. Sci.*, 28, 1479–1494 (1971).

Sources of magnetic anomalies on the Mid-Atlantic Ridge

STRONGLY magnetised basalts occur at the basement surface in ridge crest areas (see, for example, refs 1, 2) and various models for the magnetic sources have been devised. A uniformly magnetised layer, extending downwards from the basement surface for 400–500 m is consistent with most of the geophysical information, although some older estimates and the low magnetisation of some sets of rocks³ require a layer thickness of as much as 2.5 km. During Leg 37 of the Deep Sea Drilling Project⁴ five basement holes, all deeper than 100 m, were drilled from DV Glomar Challenger along a seafloor spreading flow line from the median valley of the Mid-Atlantic Ridge in the project FAMOUS survey area into the American Plate (Fig. 1). Basement penetration ranged from 108 m to 582 m and totalled 1,453 m with 243 m recovery. Here we report the results of a shipboard palaeomagnetic reconnaissance of 295 vertically oriented basement samples.

At the two oldest sites, 334 at 9 Myr and 335 at 11–16 Myr, the uppermost basement sections consist of monotonous rather massive pillow basalts which are strongly magnetised with sharply defined inclination ranges (Fig. 2). At site 334, located

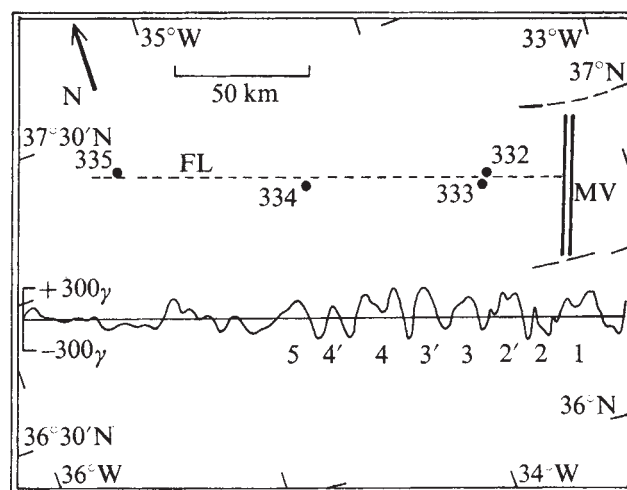
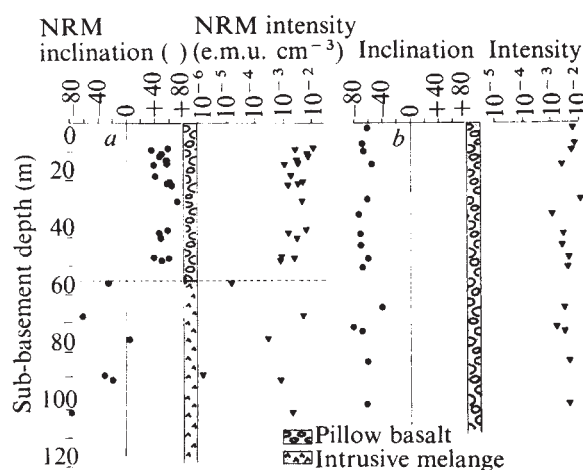


Fig. 1 Location of DSDP Leg 37 sites along a seafloor spreading flow line (FL) to the west of the Median Valley (MV) of the Mid-Atlantic Ridge. The magnetic anomaly profile along the flow line is shown with the anomalies numbered following Talwani *et al.*⁵

within positive magnetic anomaly 5, 55 m of normally magnetised basalts (mean inclination ($\pm$ s.d.m.) for 18 samples, $\bar{I}$, of $54^\circ \pm 2^\circ$) overlie a mélange of gabbros and serpentinised peridotites with scattered but generally upward magnetic inclinations. Here and at site 335 (108 m of basalt penetrated, with 15 samples yielding $\bar{I} = 65 \pm 1^\circ$) basalt magnetic polarity matches the sense of the magnetic anomaly at the site.

But at sites 332 and 333, both with an estimated basement age of 3.5 Myr, and located within the negative anomaly between 2' and 3' corresponding to part of the Gilbert reverse polarity epoch, the basement lithological and magnetic sequences (Fig. 3) are complex with rapid vertical and lateral variation. Extrusive and possibly intrusive types of basalt occur, together, in the uppermost 300 m of basement, with very extensive zones which, because they drill very rapidly with negligible recovery, are interpreted as consisting of basalt rubble and sediment. In the 333-m thick basement section of hole 332A an 80-m thick normal (N) polarity zone overlies a 130 m thick dominantly reverse (R) polarity zone. The lowest 120 m of this hole, represented by 35 samples from at least 10 cooling units, is dominated by magnetic inclinations close to the horizontal. These shallow ($+20^\circ \geq I \geq -20^\circ$) anomalous inclinations are found extensively in all three deep basement holes in this area (332A, 332B and 333A). Hole 332B, situated only 100 m laterally from 332A, and penetrating basement to a

Fig. 2 NRM inclination and intensity profiles based on individual sample values for: a, site 334; b, site 335.



depth of 582 m, has a markedly different inclination profile. The 80-m thick N polarity zone at the top of the basement section in hole 332A is missing. Although visually similar, distinctive coarse grained plagioclase-phyric basalts cap the basement sections in both holes, and the polarities are opposite being N in 332A and R in 332B. The differences between the 332A and 332B basement sections are surpassed by those between 332B, 332C and 332D, the last two only sampling the uppermost basement unit during attempts to re-enter hole 332B. All three holes are located within 10 m of each other and the topmost basement unit at 332C is the same reversely magnetised, coarsely plagioclase-phyric basalt as at 332B, but the topmost unit at 332D is a strongly normally magnetised aphyric basalt.

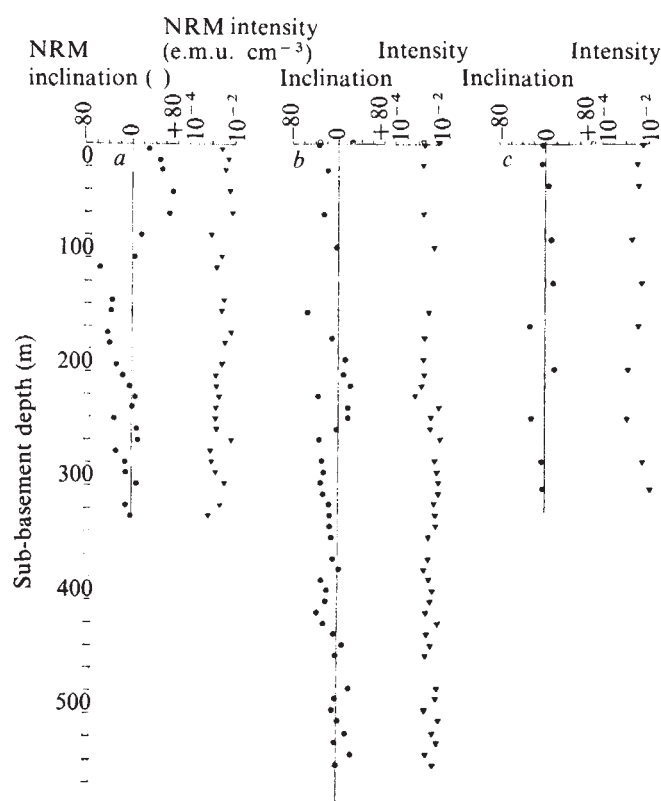
Any model for basement formation at site 332 requires accumulation during at least two geomagnetic polarity epochs of opposite senses. These may be contiguous epochs if the sequence consists of superimposed lavas, or widely separated if intrusives are present.

An important result for anomaly generation is the absence of any overall trend with depth in the magnetic intensity of the basalts (Figs 2 and 3). Magnetic intensity does change rapidly over small vertical intervals, but this can be attributed to petrological variation and degree of alteration. Thus, no direct evidence is available on the question of the nature of the lower limit of a magnetic source layer.

Modelling, using the representative values of magnetic intensity now available for each site, gives an alternative method of estimating magnetic source thickness.

At the two older sites, 334 and 335, the average basalt magnetic intensity, giving unit weight to mean values for each 9.5 m core length, is close to 40×10^{-4} e.m.u. cm^{-3} , or 32×10^{-4} e.m.u. cm^{-3} , if allowance is made for the presence of effectively non-magnetic randomly oriented basalt rubble and essentially non-magnetic sediments. With these intensities and the observed steep inclinations, a magnetic source layer extending downwards from the basement surface for from 570 m to 820 m will suffice to produce the observed anomalies.

Fig. 3 NRM inclination and intensity profiles based on 9.5 m core length average values for: a, site 332A; b, site 332B; c, site 333.



This range in thickness extends from values comparable with previous estimates to about a factor of two more.

Although these estimates of the thicknesses of magnetic anomaly source layers are based on more representative information than all previous estimates, they still cannot be taken as generally applicable, as is clear from the presence of only 55 m of basalt at site 334 and the complex lithological and magnetic situations at sites 332 and 333. The lack of a thick basaltic magnetic source layer at site 334 is surprising since the site is well within a clear example of magnetic anomaly 5, an anomaly that, after the central positive anomaly 1, is usually the easiest to recognise on all spreading ridge systems.

At sites 332 and 333 the main magnetic sources required to produce the anomaly pattern are not present in the sections drilled, which extend to 582 m below the basement surface. Although occasional highly magnetic basalt samples (up to 175×10^{-4} e.m.u. cm^{-3}) were found throughout the basement sections, the combination of the presence of abundant basalt rubble and sediment, together with commonplace shallow inclinations and polarity reversals (Fig. 3) reduces the effective magnetisation of these sections to an insignificant level. Only a 57-m thick sequence of massive picritic pillow basalt in hole 332B, physically akin to the sites 334 and 335 basalts, and with its top at close to 270 m sub-basement, is potential anomaly source material. The average magnetic properties, giving unit weight to mean values for each 9.5 m core length, for the basement sections of holes 332A, 332B and 333A are intensity 39×10^{-4} e.m.u. cm^{-3} (or 21×10^{-4} e.m.u. cm^{-3} if allowance is made for non-magnetic material) and inclination -04° . A magnetic anomaly source layer with these properties would have to be in excess of 2.5 km in thickness to produce the observed anomalies. This figure exceeds the local thickness of volcanic layer 2. Modelling using an inclination of -04° for the reversely magnetised block in which sites 332 and 333 are located, and $\pm 60^\circ$ elsewhere (implying that the reversed anomaly is effectively produced by a gap in a normally magnetised layer), with a source layer thickness of 570 m throughout, produces a poor fit with observation in the vicinity of sites 332 and 333. To obtain a satisfactory fit it is necessary to assume either that the shallow inclination magnetisation of the top 600 m of layer 2 has an average azimuth well away from the present magnetic north-south direction, or that the magnetic source layer is confined to depths in excess of 600 m sub-basement. Lack of trends with depth in basalt magnetic intensity allow for the latter possibility.

We thank Captain J. C. Clarke, the crew, drilling and laboratory teams on Glomar Challenger for help. Patrick Ryall of Dalhousie University computed magnetic source layer thicknesses using programs supplied by David Ross and S. P. Srivastava. Dalhousie University and the National Research Council of Canada equipped the shipboard palaeo-magnetic laboratory. The Deep Sea Drilling Project is funded by the National Science Foundation and managed by the Scripps Institution of Oceanography.

SCIENTIFIC PARTY DEEP SEA DRILLING PROJECT LEG, 37*

*James M. Ade-Hall, F. Aumento, Department of Geology; R. D. Hyndman, Institute of Oceanography, Dalhousie University, Halifax, Nova Scotia, Canada; W. G. Melson, Department of Mineral Sciences, National Museum of Natural History, Smithsonian Institution, Washington, DC; Henri Bougault, Centre Oceanologique de Bretagne, Brest, France; Leonid Dmitrev, Institute of Geochemistry, Academy of Sciences of the USSR, Moscow, USSR; Joseph Fischer, Department of Geology, University of Texas, Arlington, Texas; Paul T. Robinson, Department of Geological Sciences, University of California, Riverside, California; Thomas L. Wright, United States Geological Survey, Washington, DC; Gregory A. Miles, Department of Geology, University of Oregon, Eugene, Oregon; Martin T. Flower, Institut für Mineralogie, Ruhr-Universität, Bochum-Querenberg, West Germany; Robert C. Howe, Department of Geology and Geography, Indiana State University, Terre Haute, Indiana.

Received November 19, 1974; accepted April 1, 1975.

- ¹ Ade-Hall, J. M., et al., *Can. J. Earth Sci.*, **10**, 679-696 (1973).
- ² Fox, P. J., and Opdyke, N. D., *J. geophys. Res.*, **78**, 5139-5154 (1973).
- ³ Lowrie, W., Lovlie, R., and Opdyke, N. D., *Earth planet. Sci. Lett.*, **17**, 338-349 (1973).
- ⁴ Melson, W. G. et al., *Geotimes*, **19**, part 12, 16-18 (1975).
- ⁵ Talwani, M., Windisch, C. C., and Langseth, M. G., *J. geophys. Res.*, **76**, 473-517 (1971).

Geological interpretation of whole-rock isochron dates from high grade gneiss terrains

MANY Rb–Sr whole-rock isochron dates have been reported on extensive terrains of quartzo–feldspathic orthogneisses. Most are of Precambrian age. In most cases the date is either regarded simply as the “age” of the gneiss complex (with no specific interpretation), or as the age of regional metamorphism. The latter interpretation naturally implies large scale regional homogenisation of Sr isotopes at the isochron date. I find such an interpretation improbable.

First, good “primary” Rb–Sr whole-rock isochrons are commonly observed in gneiss terrains which underwent much later intense metamorphism (at least up to amphibolite facies) indicating isochemical behaviour with respect to Rb and Sr on a whole-rock scale. Thus, Scourian gneisses of north-west Scotland yield Rb–Sr whole-rock dates of about 2,700 Myr, in spite of being subjected to intense Laxfordian metamorphism and deformation nearly 1,000 Myr later, as shown by mineral dates^{1,2}. Similarly, the Amitsoq gneisses of West Greenland have preserved their 3,700 Myr Rb–Sr whole-rock isochron systematics in spite of amphibolite facies metamorphism and intense deformation at about 2,600–2,800 Myr ago^{3,4}. Many analogous cases have been reported.

Second, Krogh and Davis⁵ have demonstrated unequivocally from Rb–Sr laboratory studies on gneisses of the French River area of the Grenville Province of Ontario that isotopic and chemical equilibrium was not reached over distances of even a few centimetres during a regional amphibolite facies metamorphism which occurred some 800 Myr after their “formation”.

Why, then, do extensive gneiss terrains frequently yield such good Rb–Sr whole-rock isochrons, and what do these isochrons really mean? I have suggested that low, upper mantle type, initial ⁸⁷Sr/⁸⁶Sr ratios of many ancient gneisses indicate that their immediate precursors are predominantly juvenile additions to the continental crust at, or close to, the measured age⁶. The interval between times of extraction of juvenile igneous material from the upper mantle and/or subducted basic lithosphere and time of production of the regional metamorphic characteristics of the derived gneiss complex may be less than 50–100 Myr, which falls within the analytical uncertainty of most age measurements on Precambrian rocks. In my model major crustal accretion is regarded⁶ as essentially contemporaneous with the profound geochemical and petrological differentiation required to yield a compositionally gradational crust, in which the raw materials of granite (*sensu lato*), as well as geochemically incompatible elements and water, migrate upwards, leaving behind depleted granulite facies rocks at depth^{7,8}.

On such a model, Rb–Sr whole-rock isochrons on orthogneisses simply give the age of the accretion–differentiation event. The best isochrons are obtained when the duration of the latter event is short in relation to its actual age. A low, homogeneous, initial ⁸⁷Sr/⁸⁶Sr ratio is inherited directly from the upper mantle or basic lithosphere source region, while local and regional dispersion in Rb/Sr ratios within the gneiss complex results from synchronous geochemical differentiation processes. Significant deviations from ideal Rb–Sr isochron behaviour are quite common, and may be attributed to source region heterogeneities, to interactions of the gneiss precursors with pre-existing crust, or to lack of closed system behaviour on a whole-rock scale with respect to Rb and/or Sr during subsequent metamorphism and tectonism.

These considerations also apply to Pb–Pb whole-rock isochron dates on ancient orthogneisses, which are frequently in good agreement with corresponding Rb–Sr dates^{2,9,10}. In such cases, simplicity of the Pb–Pb isotope systematics suggests derivation from a homogeneous U–Pb (usually expressed as ²³⁸U/²⁰⁴Pb) source region approximating to single stage evolu-

tion from time of formation of the Earth to the measured isochron date¹¹, although small deviations from ideal behaviour are common. Consideration of the actual ²³⁸U/²⁰⁴Pb value of the source region (about 7.3–8.0, using the model parameters of Oversby¹²) as calculated from the primary Pb isotope growth curve and of the customarily close agreement of a given Pb–Pb isochron date with that obtained from the intersection of the Pb–Pb isochron with the primary growth curve, suggests that the source region of the gneiss precursors was upper mantle or basic lithosphere.

Synchronous geochemical differentiation of newly accreted sial produces severe U–Pb fractionation at the measured isochron date¹³. The wide range of measured ²³⁸U/²⁰⁴Pb values in gneisses contrasts strongly with the homogeneous ²³⁸U/²⁰⁴Pb value calculated for the source region of the gneiss precursors. Profound uranium depletion is characteristic of many gneisses, yielding present-day unradiogenic Pb isotope ratios close to the primary growth curve^{10,11}.

By close analogy with the Rb–Sr system, homogeneity of initial Pb isotope ratios in a gneiss terrain is thus regarded as being inherited from the source region of the gneiss precursors, whereas geochemical differentiation results in dispersion of U–Pb ratios. The necessary conditions for isochron behaviour may thus be met. A good Pb–Pb whole-rock isochron can date the primary accretion–differentiation event, which is usually constrained to a time interval of not more than about 50–100 Myr.

The above interpretation of Rb–Sr and Pb/Pb whole-rock isochron dates in ancient quartzo–feldspathic orthogneisses obviates the necessity for invoking the popular, but highly implausible, process of large scale homogenisation of Sr and Pb isotopes throughout extensive volumes of metamorphic basement rocks.

S. MOORBATH

Department of Geology and Mineralogy,
Oxford University, Oxford, UK

Received April 7; accepted April 24, 1975.

- 1 Lyon, T. D. B., Pidgeon, R. T., Bowes, D. R., and Hopgood, A. M., *J. geol. Soc. Lond.*, **129**, 389 (1973).
- 2 Moorbath, S., Powell, J. L., and Taylor, P. N., *J. geol. Soc. Lond.*, **131**, 213 (1975).
- 3 Moorbath, S., O'Nions, R. K., Pankhurst, R. J., Gale, N. H., and McGregor, V. R., *Nature phys. Sci.*, **240**, 78 (1972).
- 4 Pankhurst, R. J., Moorbath, S., Rex, D. C., and Turner, G., *Earth planet. Sci. Lett.*, **20**, 157 (1973).
- 5 Krogh, T. E., and Davis, G. L., *Yb. Carnegie Instn Wash.*, **72**, 601 (1973).
- 6 Moorbath, S., *Nature*, **254**, 395 (1975).
- 7 Fyfe, W. S., *Phil. Trans. R. Soc.*, **A273**, 457 (1973).
- 8 Heier, K. S., *Phil. Trans. R. Soc.*, **A273**, 429 (1973).
- 9 Rosholt, J. N., Zartman, R. E., and Nkomo, I. T., *Bull. Geol. Soc. Am.*, **84**, 989 (1973).
- 10 Black, L. P., Gale, N. H., Moorbath, S., Pankhurst, R. J., and McGregor, V. R., *Earth planet. Sci. Lett.*, **12**, 245 (1971).
- 11 Moorbath, S., Welke, H., and Gale, N. H., *Earth planet. Sci. Lett.*, **6**, 245 (1969).
- 12 Oversby, V. M., *Nature*, **248**, 132 (1974).
- 13 Moorbath, S., in *The Early History of the Earth* (edit. by Windley, B. F.) (J. Wiley, in the press).

Depths of origin of Kenyan basalts and implications for the Gregory Rift

THE Gregory Rift of Kenya, part of the eastern branch of the Afro–Arabian rift system, has received much attention in recent years as an exceptionally well exposed example of an intracontinental rift. It exhibits a variety of lavas and ignimbrites related to one another in time and space in patterns that can be clearly defined^{1–3}. Explicit models of the crust and upper mantle in and near the Gregory Rift, have been derived, largely from gravity surveys^{4,5}.

Two petrogenetic models for suites of basalts found in and near the Gregory Rift have been developed. One of these models, for the Chyulu Basalts found east of the rift, overlying Precambrian metamorphic rocks, suggests that the parental basalts of this suite effectively equilibrated with mantle rocks at a temperature of ~1,450 °C and pressures of substantially less than 25 kbar⁶. Analytical data on abundances of rare earth elements in the Chyulu Basalts show that assimilation of crustal materials has had at most a very minor effect on the compositions of these rocks, suggesting that the magmas they represent

had not remained in contact with crustal rocks for very long. The other model, for members of a suite including a variety of basalts collected in the southern part of the Gregory Rift near Ologresailie, an area in which the volcanic rocks are predominantly trachytes, suggests that parental basalts of the suite fractionated to produce the more-evolved basalts at temperatures of about 1,200 °C or slightly greater and at pressures of roughly 3–10 kbar (unpublished).

Given the compositions of these basalts, a means of estimating densities of magmas of corresponding compositions at appropriate temperatures and pressures, and models of the densities of rocks from the crust and upper mantle of the region, it is a simple matter to estimate the depths of origin of the magmas if it is assumed that conditions approximating to hydrostatic equilibrium exist. Such estimates provide insight into the structure and geological evolution of the Gregory Rift.

I have taken the compositions of Chyulu Basalt KCH-1 and Gregory Rift Basalt KLR-32 (ref. 6) and computed the densities of liquids with equivalent compositions (according to the method of Bottinga and Weill⁷ at 1,400 °C and at 1,250 °C, respectively. The procedure for this is straightforward except that the effects of P_2O_5 and H_2O must be taken into account. I estimated partial molar volumes of P_2O_5 by comparing molar volumes of berlinite and corundum⁸, and arrived at a value of about 70 cm³ mol⁻¹ at 1,250 °C and a slightly larger value at 1,400 °C. These estimates are crude but the effect of P_2O_5 on the densities of the liquids is small because of the minor contents of this oxide in these basalts.

The effects of H_2O on the densities are, however, more marked and, because of loss of H_2O from the basalts during eruption and crystallisation, more difficult to assess. There is a high proportion of pyroclastic materials among the products of the Chyulu vents, but there is no evidence for such materials related to the Gregory Rift Basalts treated here. Consequently, it seems likely that the initial H_2O contents of the Chyulu magmas were substantially higher than were those of the KLR basaltic magmas. I have arbitrarily assumed that the initial H_2O content of the KCH-1 magma was 1.0% by mass, and that the initial H_2O content of the KLR-32 magma was 0.5% by mass.

Preliminary calculations indicated that, in agreement with conclusions drawn from petrogenetic models cited already, the Chyulu magmas probably arose from depths great enough to necessitate the application of a small correction for the effects of pressure on the average density, but no correction is necessary for the liquid representing the KLR-32 magma. The estimated densities are 2.71 g cm⁻³ (Chyulu liquid, with pressures ranging from 0–12 kbar) and 2.65 g cm⁻³ (Gregory Rift liquid).

These densities are lower limits for the magmas which gave rise to the analysed basalts; the presence of crystals would tend to increase the magma densities above those estimated under the assumption that only liquid was present. Both basalts are, however, nearly aphyric, and consequently the estimates of densities should not be significantly in error from this cause.

Darracott, Fairhead, and Girdler⁵ have devised a model for densities of the lithospheric rocks at various depths near the Gregory Rift: 2.67 g cm⁻³ from 0 to 4 km; 2.85 g cm⁻³ from 4 to 17.5 km; 2.90 g cm⁻³ from 17.5 km to the base of the crust at 36.2 km; and 3.34 g cm⁻³ in the upper mantle down to the base of the lithosphere at about 90 km. The highest peaks of the Chyulu Hills are about 1.4 km above the general level of the surrounding Precambrian rocks. This elevation is probably close to, though somewhat less than, the excess hydrostatic head, h , which governed the eruptions that built up these cinder cones. If h were about 2 km, a column of magma with average density of 2.71 g cm⁻³ would have been in hydrostatic equilibrium with rocks of the model of Darracott, Fairhead, and Girdler at a depth of about 37 km (that is, slightly below the base of the crust), in agreement with the

inferences drawn from the petrogenetic model for the Chyulu Basalts⁶. If the volatile content of such a magma column were less than that assumed, or if there were appreciable proportions of crystals suspended in it, its average density would be higher so that h would have to decrease, assuming a constant depth of origin. For instance, if the average density were 2.79 g cm⁻³ as estimated for a volatile-free liquid corresponding to KCH-1, h would be about 1 km if the depth of origin was 37 km. Alternatively, one could assume a slightly greater depth of origin and constant h to account for the effects of a higher density.

These results provide support for the conclusion that the Chyulu magmas originated at unusually high temperatures and (for alkaline basalts) low pressures, and also increase confidence in the model of Darracott, Fairhead, and Girdler⁵ and in the Bottinga and Weill⁷ method for estimating magma densities.

Consider applications of similar arguments to the basalts of the Gregory Rift near Ologresailie. If the KLR-32 magma, with a predicted density of 2.65 g cm⁻³, erupted from a depth of origin, h , of 1 km, it could have been in hydrostatic equilibrium with its wall rocks as represented by the model of Darracott, Fairhead, and Girdler⁵ only if it had its roots about 13 or 14 km below the rift floor. Probably, KLR-32 does not represent a relatively unmodified mantle-derived magma, but instead was stored (and presumably fractionated) in a shallow magma chamber within the axial zone of the rift before eruption.

Some characteristics of this putative, shallow magma chamber can be inferred from the results of geophysical studies of the rift. In 1970, Searle (ref. 9) modelled the gravity anomaly along the axis of the central Gregory Rift as a large basic intrusion, with a density of 2.90 g cm⁻³ at depths of less than about 25 km, which penetrates to within about 3 or 4 km of the floor of the rift. This conclusion was accepted in slightly modified form by subsequent workers^{4,6}, and it leads to a plausible model for the tectonics of the rift. Of course, there is no reason to believe that this dense intrusive body was produced by the crystallisation of a single, large magma reservoir rather than by crystallisation of a series of smaller magmatic injections, nor does the geophysical evidence require that the intrusive body be in contact with the upper mantle.

If magma which crystallised to form a part of this intrusive body were present in the axial zone of the Gregory Rift near Ologresailie at the time of the eruption of KLR-32 about 1.6 Myr ago, it should have been in hydrostatic equilibrium with its wallrocks. Fixing its top at 3 km below the rift floor and assuming that it was in effective contact with wallrocks like those modelled by Darracott, Fairhead and Girdler, its density must have been appreciably greater than that estimated for the KLR-32 magma; it perhaps resembled the KCH-1 magma in that respect. It cannot have had vertical dimensions greater than about 10–20 km: had that been the case eruptive basaltic volcanism would have been much more common in the area at that time. This magma may have been a thin veneer on top of dense intrusive rocks representing the solidified remains of similar, older magmas related to previous cycles of basaltic volcanism (the Singaraini, Kordyja, and Kirikiti basalts).

It seems that we are dealing with something like a bubble of magma, or a series of such bubbles, drawn off from the upper mantle either in response to, or as a cause of, rifting and confined to the axial zone of the rift, but of limited vertical extent. Note that the crustal thickness of about 36 km in this region would have allowed a much bigger bubble to be confined in the axial zone of the rift. A bigger bubble would necessarily have manifested itself in a phase of extensive basaltic effusive volcanism, as is observed further north in the Gregory Rift^{1–3}. Probably, the distinction between portions of the rift in which basaltic flows predominate and those in which basalts are uncommon and trachytic or phonolitic flows predominate is simply a reflection of different vertical dimensions of the axial bubbles, which are in turn related either to the volume of

basaltic magma that may be drawn off from the underlying upper mantle or to the propensity of the mantle for giving up its magma in one large batch or in two or more smaller batches.

Presumably, contrasts in the volume of basaltic magma that may be derived in a short interval from a given area of the upper mantle under the rift are related to the fertility of the mantle, with those portions of the upper mantle that have previously been physically depleted being incapable of generating enough basaltic magma, or of generating it fast enough, to provide a big bubble. In this context, it is interesting that basaltic volcanism seems to die away as the rift continues into the African continent. To the degree that the suboceanic upper mantle participates in the plate tectonic cycle, it may be replenished with the components that are required for the generation of basaltic magma and so remain capable of producing magma, but subcontinental upper mantle that is part of the lithosphere may be more or less isolated from the plate tectonic cycle and so rather easily becomes depleted of magma-producing constituents. Perhaps the different styles of volcanism at different locations along the Gregory Rift reflect a broad transition from one kind of upper mantle to another. These speculations may be tested, *inter alia*, by studies of Sr and Pb isotopes in rocks collected from portions of the Gregory Rift which show different styles of volcanism.

GORDON G. GOLES*

Nuclear Physics Research Unit,
University of the Witwatersrand,
Johannesburg, South Africa

Received and accepted April 9, 1975.

*Center for Volcanology, University of Oregon, Eugene, Oregon 97403.

¹ Williams, L. A. J., *Bull. Volcanol.*, **34**, 439–465 (1970).

² Baker, B. H., Williams, L. A. J., Miller, J. A., and Fitch, F. J., *Tectonophysics*, **11**, 191–215 (1971).

³ King, B. C., and Chapman, G. R., *Phil. Trans. R. Soc. A*, **A271**, 185–208 (1972).

⁴ Baker, B. H., and Wohlenberg, J. G., *Nature*, **229**, 538–542 (1971).

⁵ Darracott, B. W., Fairhead, J. D., and Girdler, R. W., *Tectonophysics*, **15**, 131–141 (1972).

⁶ Goles, G. G., *Lithos*, **8**, 47–58 (1975).

⁷ Bottinga, Y., and Weill, D. F., *Am. J. Sci.*, **269**, 169–182 (1970).

⁸ Robie, R. A., Bethke, P. M., and Beardsley, K. M., *Bull., U. S. geol. Surv.*, **1248** (1967).

⁹ Searle, E. C., *Geophys. J. R. astr. Soc.*, **21**, 13–31 (1970).

Shatter cones from Houghton Dome, Devon Island, Canada

SHATTER CONES, products of shock metamorphism, have been discovered at Houghton Dome and indicate a meteorite impact origin for this circular structure. Houghton Dome is centred at 89°40'W, 75°22'N on Devon Island in the Canadian Arctic Archipelago. Although structurally a dome, it forms a topographic depression incised on the regional plateau (Fig. 1). The inner, circular basin, 17–18 km diameter, has an average elevation of 140 m above sea level compared with regional elevations of 340–400 m. It is surrounded by an annular ridge with elevations averaging 270 m above sea level. In the southern portion of the structure the ridge is not pronounced, but forms a shoulder on the gradual upward slope to the regional plateau, approximately 17 km from the centre. Streams draining the basin have dissected the ridge in the northern portion into an arc of isolated hills. Steep-walled drainage valleys outside the ridge are circumferential or tangential to the structure and enhance its circular appearance.

The Precambrian basement of Devon Island is exposed 100 km to the east, and its surface forms a gently westerly dipping homocline¹. Exposures of the overlying sedimentary sequence proceed in westerly succession from Cambrian (?) to Devonian (?) with the Allen Bay Formation (Upper Ordovician to Middle Silurian) forming the plateau in the Houghton Dome region. Dolomite, limestone and gypsiferous

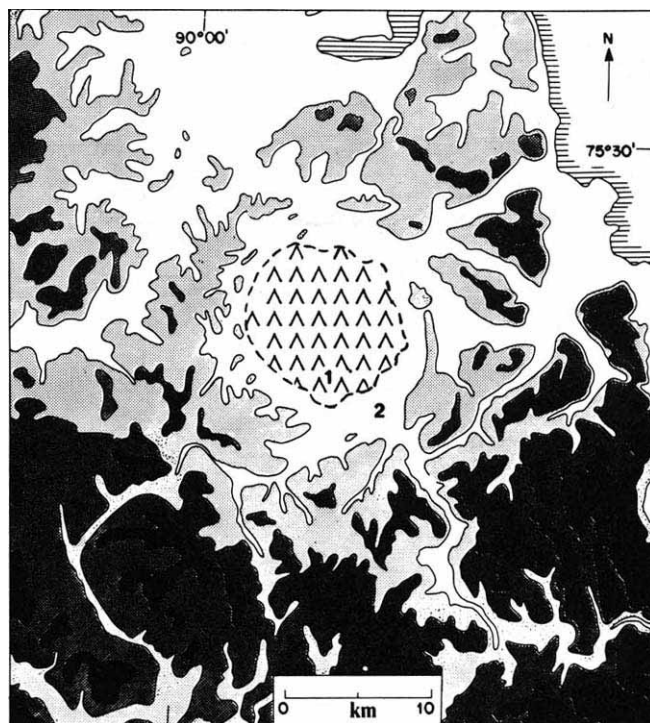


Fig. 1 Topographic map of the Houghton Dome structure showing central depression and partial annular ridge. Morphology of the structure is enhanced by leaving unshaded elevations below 800 feet (244 m) above sea level. Thereafter, shading darkens with each 200 feet (61 m) increase in elevation. Horizontal shading is Thomas Lee Inlet. AAA, Approximate outcrop area of the evaporite unit. Shatter cones were collected in limestone rubble at location 1, but were not found at 2.

and dolomitic shales of the Cornwallis Group (M. Ord.) underlie the Allen Bay limestones and are exposed at the base of cliffs along Thomas Lee Inlet and in river valleys 10–15 km east of Houghton Dome, where beds dip westward at less than 2°.

The geology of Houghton Dome is partially known from mapping in the south-east quadrant², supplemented by aerial reconnaissance and examination of aerial photographs. Exposed in the central depression are limestones and evaporites which form white cliffs 60–80 m high. Greiner² observed complex folds and zones of shearing in this area. The central depression is surrounded by the following succession, as mapped in the south-east²: a zone of brecciated limestone; a zone of "complex structures and lithology" which may form the annular ridge; limestones and dolomites in a series of shallow anticlines and synclines with axes tangential to the structure at approximately 10 km from the centre. Correlation between regional stratigraphy and disturbed rock units within the structure is tenuous. Greiner² considered the evaporites to be the lower part of the Cornwallis Group, which does comprise a thick evaporite section on islands 140 km west and north¹, but not in this part of Devon Island. More probably, from their weathering characteristics, the evaporites may correlate with the Lower Ordovician Baumann Fiord Formation which is widespread in the eastern Arctic islands³, a formation which was not known when Greiner made his correlation. Limestones and dolomites of the marginal zones include Cornwallis and Allen Bay units, and possibly the Eleanor River Formation which separates the Baumann Fiord and Cornwallis Group. Because of the circular exposure of evaporite surrounded by younger disturbed rocks, Houghton Dome has been considered a piercement dome¹, especially as many piercement domes are emplaced in the Sverdrup Basin to the north⁴. These structures are, however, smaller and form a distinct cluster in a different tectonic setting 400 km distant.

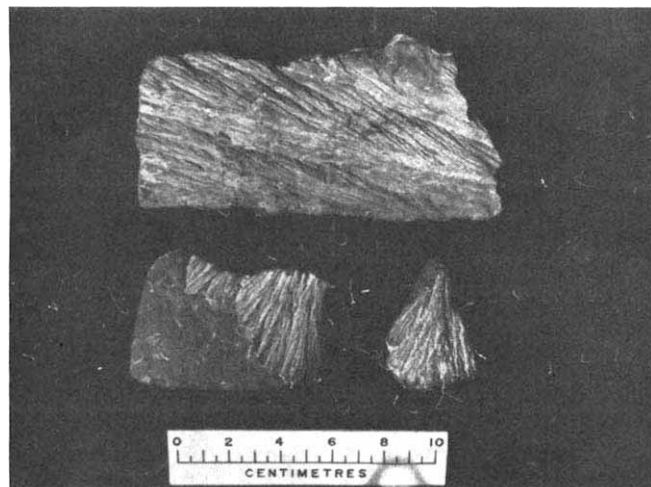


Fig. 2 Shatter cone fragments in fine-grained limestone. Other fragments indicate cones at least 30 cm high.

Shatter coning and intense microtwinning of calcite, present in samples from Haughton Dome, are indicators of shock metamorphism. Shatter cones are abundant in limestone rubble at the one locality visited within the central depression (Fig. 1). Their extent has not been determined although shatter cones were not found at a locality in the outer brecciated limestone zone. Most of the samples collected (by G.D.M.) are broken, partial cone surfaces (Fig. 2) with 110° the maximum arc found intact. Shatter cones on surfaces where apex and base are seen are generally 4–7 cm high, whereas surface curvature and convergence of striations on broken segments indicate cones at least 30 cm in height. The characteristic horsetail ridges are finely-developed on the fine-grained limestones, although erosion or a skin of calcite plus aragonite commonly subdues or masks all but the coarser striations.

Shatter cones are accepted as products of hypervelocity impact because of their occurrence only at meteorite craters and astroblemes⁵. They are formed by tensional release in an axially symmetrical field following compression by an expanding shock wave⁶ with peak pressures in excess of approximately 2 GN m^{-2} ($1 \text{ Gnewton m}^{-2} = 10 \text{ kbar}$). The absence of shatter cones at locality 2, 8 km from the centre, is consistent with their disappearance at approximately one-half radius in large impact structures⁷.

Calcite in the shatter-coned limestones shows intensive microtwinning with up to six sets per grain. The twin planes, $(01\bar{1}2)$ and $(10\bar{1}1)$, are those commonly produced in tectonic deformation, but their close spacing, $<20 \mu\text{m}$, is consistent with shock metamorphism as illustrated in shatter-coned limestones at the Flynn Creek crater, Tennessee⁸. Shock-induced planar features were not observed in the minor amounts of detrital quartz grains within the Haughton Dome limestone samples.

Haughton Dome can be classed as an eroded, complex, hypervelocity impact structure with a central structural uplift⁹. It can be compared with Sierra Madera (12 km) and Wells Creek (13 km) structures, formed largely in carbonate rocks. At these two impact sites material in the central uplift has been raised 1,200 m (ref. 10) and 780 m (ref. 11) respectively. Based on average stratigraphic thicknesses and regional dips, uplift at Haughton Dome is approximately 400 m (if Cornwallis evaporite in centre) to 1,500 m (if Baumann Fiord evaporite in centre). The latter figure, of a magnitude more consistent with uplift at Sierra Madera and Wells Creek, tends to support a correlation of the central evaporite with the Baumann Fiord Formation.

From stratigraphic constraints the maximum age of the Haughton Dome impact is 425 Myr (that is younger than the

Allen Bay Formation) although it predates Pleistocene glaciation.

The original suggestion of Dence⁹ that Haughton Dome is an impact structure is confirmed by the evidence of shock metamorphism in the form of shatter cones. The addition of Haughton Dome brings the number of confirmed meteorite impact structures recognised in Canada¹² to twenty-one.

P. B. ROBERTSON

Earth Physics Branch, Gravity Division,
Department of Energy, Mines and Resources,
Ottawa, Canada, K1A 0E4

G. D. MASON

Cominco Ltd, 120 Adelaide St W.,
Toronto, Canada, M5H 1T1

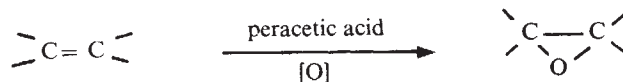
Received March 18; accepted April 16, 1975.

- ¹ Fortier, Y. O., *Mem. geol. Surv. Can.*, **320**, 156–162 (1963).
- ² Greiner, H. R., *Mem. geol. Surv. Can.*, **320**, 208–216 (1963).
- ³ Kerr, J. W., *Bull. Can. petrol. Geol.*, **15**, 91–113 (1967).
- ⁴ Thorsteinsson, R., *Econ. Geol. Rep. Surv. Can.*, **1**, 551 (1970).
- ⁵ Dietz, R. S., *Int. geol. Cong. Session 24, Proc. Section 15*, 112–118 (1972).
- ⁶ Manton, W. I., *Ann. N.Y. Acad. Sci.*, **123**, 1017–1049 (1965).
- ⁷ Robertson, P. B., *Meteoritics*, **4**, 89–112 (1968).
- ⁸ Roddy, D. J., in *Shock Metamorphism of Natural Materials*, 291–322 (Mono Book Corp., Baltimore, 1968).
- ⁹ Dence, M. R., *Int. geol. Cong. Session 24, Proc. Section 15*, 77–89 (1972).
- ¹⁰ Wilshire, H. G., and Howard, K. A., *Science*, **162**, 258–261 (1968).
- ¹¹ Wilson, C. W. Jr, and Sterns, R. G., *Bull. Tenn. Div. Geol.*, **68** (1968).
- ¹² Robertson, P. B. and Grieve, R. A. F., *Jl R. astr. Soc. Can.*, **69**, 1–21 (1975).

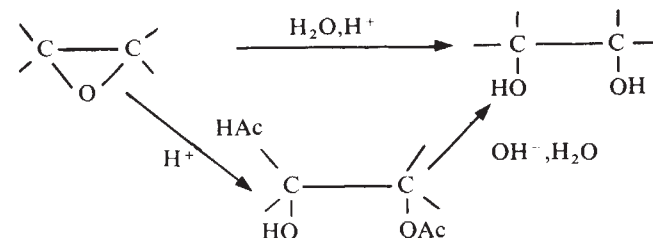
Effect of stress transfer modified swelling on diffusion in polymers

We have noticed that if certain 'immobile' triblock copolymers of the styrene-butadiene-styrene (SBS) type are subjected to surface hydroxylation by peracetic acid (a low molecular weight diffusing species) some anomalies occur in the diffusion-reaction process. We attributed these anomalies to the previously unnoticed effect of swelling in the surface region of the SBS polymer, which in turn was modified by the transfer of the swelling stresses to the interior during the reaction.

To hydroxylate SBS block copolymers in the bulk state, films of the copolymer (Shell experimental block copolymer TR-41-2443; $M_n = 118,000$; 27.7 weight % polystyrene) of various thickness were suspended in a reaction bath containing peracetic acid, sulphuric acid and varying amounts of acetic acid and water. The peracetic acid, after diffusing into the polymer, reacts with the double bonds in the polybutadiene continuous phase of the film to form an epoxide product:



The epoxide product then reacts with water or acetic acid, with sulphuric acid as a catalyst, to form, respectively, a 1,2 glycol or a hydroxyacetate addition product, the acetate group of which is hydrolysed by a base in a separate bath to give a uniform product:



The cleavage agents (including sulphuric acid) must also diffuse through the polymer to effect the ring opening of the epoxide groups.

The diffusion of all species into the polymer is governed by their respective diffusivities and solubilities in the polymer. On epoxidation, the polymer is converted from a hydrophobic to a

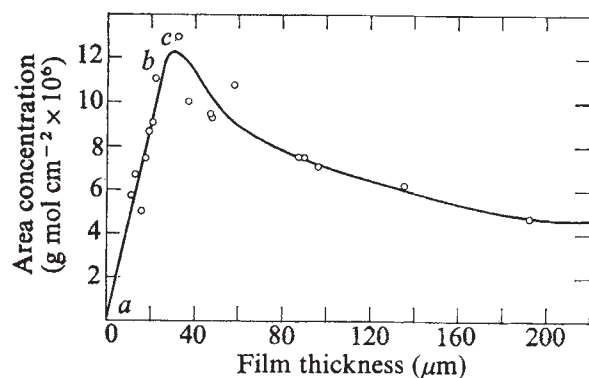


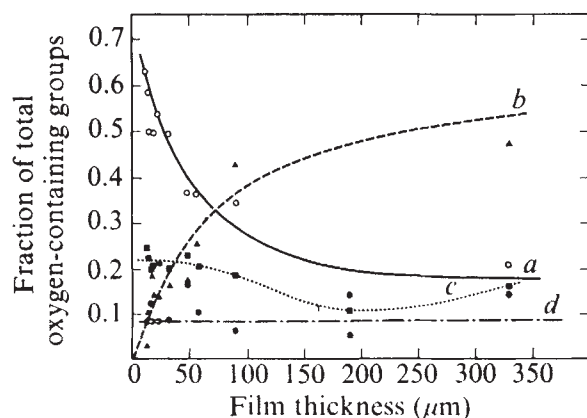
Fig. 1 Area concentration of hydroxyl against film thickness; 30 °C, 180 min, 40% acetic acid. The area concentration of hydroxyl groups, measured by infrared spectroscopy of intact reacted film, is defined as $\int_0^b C_{OH}$ (b = film thickness in cm; C_{OH} = local concentration of hydroxyl groups, mol cm⁻³) and is the total number of moles of hydroxyl groups present in the film per unit area.

hydrophilic material and the solubilities of the diffusing species are increased. In addition, swelling occurs simultaneously with reaction, resulting in an increase in the diffusivity and a still further increase in solubility. These increases of diffusivity and solubility are, however, modified by the presence of an elastic network in the SBS film, which acts to transfer the swelling stresses to the unreacted and, therefore, unswollen core (see ref. 1). This transfer of stress acts to reduce the degree of swelling in the surface region and limits the increases of diffusivity and solubility. As the degree of stress transfer depends directly on the relative size of the unreacted core and the reacted surface region, the thickness of the film has a strong effect on the permeation rate of peracetic acid and on the cleavage agents, acetic acid and sulphuric acid (resulting in a lowered permeation rate in thicker films than in thinner films).

Contrary to expectations the extent of reaction did not become independent of film thickness at a particular depth of penetration (Fig. 1). Similar observations—an initial linear increase in area concentration (extent of reaction) followed by a gradual decrease beyond a certain maximum value (Fig. 1)—were recorded for each set of conditions studied.

There are two reasons for the maximum at c in Fig. 1 and for the subsequent decrease. Quantitative analysis of the spectra of the films show the same decrease in hydroxyl concentration

Fig. 2 Composition of surface hydroxylated films; 30 °C, 180 min, 70% acetic acid. The concentrations are reported as the fraction $C_i/\Sigma C_i$, where ΣC_i = sum of equivalents of all oxygen containing species. C_i determined by infrared spectroscopy of ground samples of reacted films. a and $\circ$, Hydroxyl; b and Δ , epoxy; c and $\blacksquare$, cyclic ether; d and $\bullet$, acyclic ether.



and a commensurate increase in epoxide content (Fig. 2). Because of the transfer of stress to the dry interior, the surface regions of the thinner films swell more than those of the thicker films, enabling the slower diffusing epoxide cleavage agents (acetic acid, water and sulphuric acid) to diffuse more effectively into the thinner films, thus reducing the lag that exists between the advancing concentration profiles of peracetic acid and the cleavage agents. As a result, cleavage of the epoxy rings is more nearly complete in thinner films than in thicker films, and yields higher hydroxyl contents.

As the degree of reaction increases, the amount of swelling becomes sufficient to reduce the lag between the two concentration profiles to the extent that, apparently, the product distribution is no longer dependent upon the thickness of the film. Nevertheless, the hydroxyl content still decreases with increasing film thickness. We attribute this to the effect of stress-transfer modified swelling in the surface region on the depth of penetration of the epoxidation reaction itself. The lowered rate of permeation of peracetic acid in the thicker films (because of the reduced degree of swelling) results in a smaller depth of penetration of the reaction zone in thicker films, which is directly related to the lower hydroxyl content in these films even where there is no change in the product distribution.

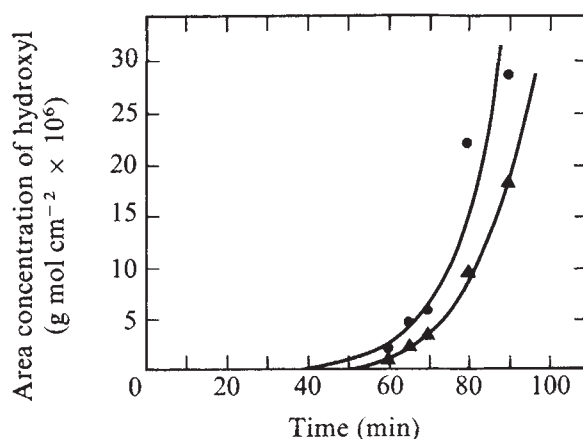


Fig. 3 Time course of surface hydroxylation. Area concentration of hydroxyl against time; 40 °C, 71% acetic acid. $\bullet$, 60 μ m; Δ , 300 μ m.

The exponential time course of the surface hydroxylation process and the apparent induction time of approximately 50 min (for a reaction at 40 °C) which lapses before there is any evidence of hydroxyl groups in the infrared spectrum are both evident in Fig. 3. The depth of penetration is, therefore, less than 0.2–0.3 μ m (as calculated from the OH absorptivity and the minimum peak area distinguishable in the spectrum) with 50 min of reaction, but is increased to a depth of the order of 20–25 μ m—a 100-increase—in just 15 min extra. This apparent induction time is related directly to the exponential nature of the process.

This exponential relationship could also be a result of swelling in the surface region. As the surface region swells, the solubility of the peracetic acid in the polymer increases and the driving force for diffusion is increased commensurately. Thus, the diffusion rate increases as the depth of penetration increases and as the degree of swelling increases at the surface because of the relief of the limiting compressive stresses. This further increases the peracetic acid solubility. This 'autoacceleration' behaviour can be verified by increasing the concentration of acetic acid in the reaction bath. Acetic acid, being a better swelling agent for the reacted polymer, should lower the length of the induction period as the same solubility should be attained at an earlier time; in fact the extent of reaction is greater after only 20 min in 93% acetic acid than it is after 65 min in the 71% acetic acid.

Surface hydroxylation is, therefore, a combined reaction limited and diffusion limited process, modified by the simultaneous swelling of the reacted surface region, which in turn is limited by stress transfer to the unreacted core. This unusual and unexpected behaviour, in which the film thickness has an effect on the diffusion and subsequent reaction of a low molecular weight permeant with the film is believed to be a general phenomenon characteristic of a variety of situations involving interfacial conversion including, for example, the oxidative photodegradation of polyethylene.

M. V. SEFTON*
E. W. MERRILL

Department of Chemical Engineering,
Massachusetts Institute of Technology,
Cambridge, Massachusetts 02139

Received December 27, 1974; accepted March 5, 1975.

* Present address: Department of Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, Canada M5S1A4.

¹ Crank, J., *J. Polym. Sci.*, 11 (2), 151 (1953).

Disorder in a hydrophobic cage illustrated by X-ray structure of α -cyclodextrin-methanol pentahydrate adduct

α -CYCLODEXTRIN (α -CD, cyclohexaamylose) is a cyclic molecule consisting of six α (1 $\rightarrow$ 4) linked glucose residues. Because of its annular structure it can form inclusion complexes with a wide variety of guest molecules even in aqueous solution and has been studied as an enzyme model¹. The structure of the 'empty' water adduct suggests a release of conformational and intramolecular hydrogen bonding energy of the α -CD molecule when complex formation occurs^{2,3}. To study this process in more detail, we have investigated the adduct with methanol.

The α -CD-MeOH adduct was crystallised through slow cooling of a saturated solution of α -CD in 50% aqueous methanol. The space group of the crystals is orthorhombic, $P2_1 2_1 2_1$, with unit cell dimensions $a = 14.339$ Å, $b = 37.365$ Å and $c = 9.465$ Å. The intensities of 4,283 reflections were measured using a STOE four circle diffractometer with Ni-filtered Cu K α -radiation and a $\theta/2\theta$ scan with a maximum 2θ of 120° .

The structure was solved by assuming it to be isomorphous with the recently published α -CD-*n*-propanol-4.8 hydrate complex⁴ which is also orthorhombic, $P2_1 2_1 2_1$ with cell dimensions $a = 14.292$ Å, $b = 37.515$ Å, $c = 9.393$ Å. The atomic parameters of the α -CD-MeOH pentahydrate structure were refined by full matrix least-squares methods to a conventional discrepancy index $R = 4.3\%$ for all the measured data. Positions of hydrogen atoms were determined from Fourier difference syntheses, hydrogen atoms bonded to disordered hydroxyl or methyl groups were not located.

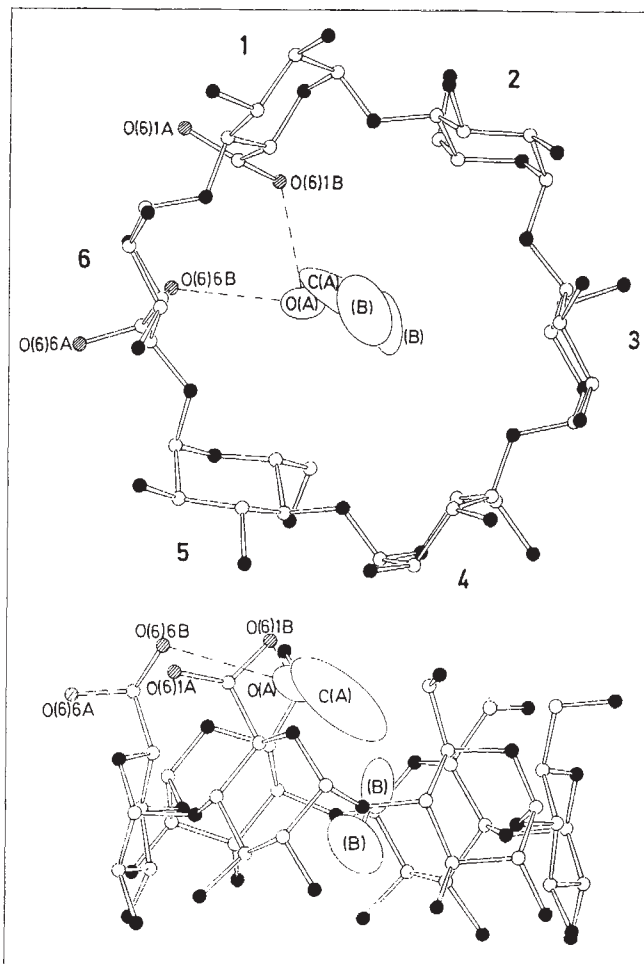
As expected, the structure of the α -CD-methanol adduct is similar to that of the *n*-propanol adduct (Fig. 1). In both the *n*-propanol and methanol adducts, atom O(6)6 (the O(6) hydroxyl group of glucose number 6) has twofold disorder, but in the methanol adduct, O(6)1 is twofold disordered as well. The methanol hydroxyl group O(A) is present at the same position as the *n*-propanol hydroxyl group in the *n*-propanol adduct with the other parts of the alcohol molecules being in different locations. The conformation of the α -CD molecule in the methanol adduct is quite similar to that in the *n*-propanol adduct. The O(4) atoms are nearly coplanar with a maximum deviation of 0.17 Å from the common mean plane similar to the *n*-propanol structure as well as the α -CD-potassium acetate⁵ and α -CD-I₂ structures⁶, but different from the strained conformation of the α -CD molecule in the water adduct^{2,3}. The α -CD macrocycle is not as symmetrical as in the *n*-propanol adduct, since the O(4) $\cdots$ O(4) distances have a wider variance, being 8.25, 8.44 and 8.66 Å, while in the *n*-propanol adduct they are 8.35, 8.47 and 8.57 Å. This discrepancy may result from

the disorder of atom O(6)1 in the methanol adduct. Both hydroxyl groups O(6)6B and O(6)1B are pointing in towards the centre of the α -CD molecule and make close contacts with O(A) of the methanol. Along this direction the molecule contracts to 8.25 Å for the O(4)3 $\cdots$ O(4)6 distance. The larger substrate molecule, *n*-propanol, apparently enables the α -CD molecule to be more symmetrical and less strained, while the smaller methanol molecule leaves α -CD slightly more strained. Going from water to methanol to propanol to potassium acetate the conformation becomes more and more symmetrical and less strained with the water adduct being substantially different from any other inclusion complex.

The two methanol molecules in the α -CD-methanol adduct are seen with half populations. The C(A)-O(A) molecule is nearly in the plane of the O(6) atoms, although C(A) has a high elongated vibrational ellipsoid. O(A) has close contacts with O(6)6B and O(6)1B as well as with O(2)1 of a neighbouring molecule. The methanol molecule (B)-(B) is nearly coincident with the axis of the α -CD molecule and is centred closer to the O(2), O(3) side. It has a high temperature factor and is held only by van der Waals' forces.

As most of the hydroxyl hydrogen atoms have been located, the hydrogen bonding scheme can be summarised. The regular hydrogen bonds between the O(2) and O(3) atoms of adjacent glucose residues are confirmed by the presence of the H atoms. The O(2) $\cdots$ O(3) distances are 3.07, 2.96, 2.91, 2.96, 2.95 and

Fig. 1 The structure of the α -cyclodextrin-methanol pentahydrate complex. The glucose numbering scheme used in the text is indicated. The two included methanol molecules O(A)-C(A) and (B)-(B) are shown with half population each and drawn with their 50% probability thermal ellipsoids. In methanol molecule A, the hydroxyl group O(A) is hydrogen bonded to O(6)6B, O(6)1B (dashed lines) while molecule B is held by van der Waals' forces and C,O atoms could not be assigned with certainty.



3.15 Å. Even for the distances longer than 3 Å the atoms were found with good O—H...O angles between 160° and 180°. Although this could not be seen in the *n*-propanol adduct as the hydroxyl atoms were not found, it is consistent with the potassium acetate adduct structure. Other than these regular features, all the hydroxyl groups O(2), O(3) and O(6) form hydrogen bonds to either water molecules or atoms of adjacent cyclodextrin molecules.

The structure of the α -CD molecule in the α -CD-MeOH complex and in the recently investigated α -CD-krypton complex⁷ is different from that in the α -CD-water complex with composition α -CD·2H₂O. With the methanol and the krypton substrate the α -CD molecule is almost hexagonal in shape and the methanol and krypton molecules are highly disordered to occupy the 5 Å wide cavity. But with the H₂O guest molecules the α -CD cavity is decreased by an inward rotation of two glucose residues to hold the two H₂O molecules in van der Waals' contact and the H₂O molecules are further fixed through hydrogen bonding. The inward rotation of the two glucose residues in the water adduct causes a conformation with increased strain energy and disrupted intramolecular O(2)...O(3) hydrogen bonds between adjacent glucoses³ and explains why in aqueous solution α -CD is able to form adducts with such a variety of guest molecules: adduct formation occurs through release of conformational strain energy of the α -CD molecule and formation of continuous O(2)...O(3) intramolecular hydrogen bonds between adjacent glucoses^{3,7}.

B.H. thanks the National Science Foundation for a NATO postdoctoral fellowship. All computations were carried out with the UNIVAC 1108 computer at the computing centre of the Gesellschaft für wissenschaftliche Datenverarbeitung, Göttingen, and were supported in part by the Deutsche Forschungsgemeinschaft.

B. HINGERTY
W. SAENGER

Max-Planck-Institut für experimentelle Medizin,
Abteilung Chemie,
34 Göttingen,
Hermann-Rein-Strasse 3, West Germany

Received February 13; accepted April 25, 1975.

¹ Griffiths, D. W., and Bender, M., *Adv. Catalysis*, **23**, 209 (1973).

² Manor, P. C., and Saenger, W., *Nature*, **237**, 392 (1972).

³ Manor, P. C., and Saenger, W., *J. Am. chem. Soc.*, **96**, 3630 (1974).

⁴ Saenger, W., McMullan, R. K., Fayos, J., and Mootz, D., *Acta Cryst.*, **B30**, 2019 (1974).

⁵ Hybl, A., Rundle, R. E., and Williams, D. E., *J. Am. chem. Soc.*, **87**, 2779 (1965).

⁶ McMullan, R. K., Saenger, W., Fayos, J., and Mootz, D., *Carbohydr. Res.*, **31**, 211 (1973).

⁷ Saenger, W., and Noltemeyer, M., *Angew. Chem.*, **13**, 552 (1974).

Continental drift and the use of albumin as an evolutionary clock

ACCORDING to the concept of proteins as evolutionary clocks¹, change in amino acid sequence during evolution is primarily a time-dependent process. This process accounts for the correlation generally found between the amount of time that has elapsed since two species last shared a common ancestor and the degree to which the sequences of their proteins differ today. A body of data consistent with such a correlation is now available for many proteins and nucleic acids²⁻⁹. Although there is increasingly widespread agreement that such a correlation exists¹⁰⁻¹² it is difficult to ascertain how strong the correlation is because of uncertainties in the interpretation of the fossil record, particularly with regard to times of divergence of lineages.

An alternative source of information about divergence times is sometimes available, even for taxonomic groups that have extremely poor fossil records: such information comes from studies of continental drift. When the land bridge between two continents disappears, many of the species on one continent become genetically isolated from their relatives on the other continent. By making intercontinental comparisons of the proteins of those taxonomic groups

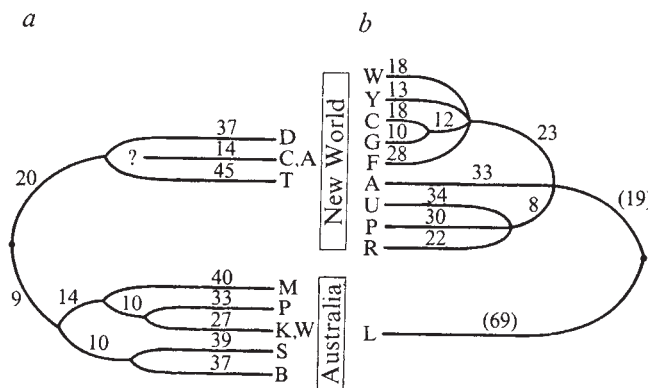


Fig. 1 Phylogenetic relationships among the albumins of marsupials (a) and hyline tree frogs (b) from Australia and the New World. For the methods of tree construction used, see refs 17, 18 and 29. By comparing immunologically each of the marsupial albumins with those of selected placentals (*Hyaena*, *Ursus*, *Genetta*), we found that *Caluromys* and *Marmosa* albumins are more conservative (that is, react more strongly with the anti-placental albumin sera), by 25 ± 5 units, than those of the other seven lineages of New World and Australian marsupials studied. The numbers above the lineages indicate the number of albumin immunological distance units of evolution calculated to have occurred along each lineage. The position of the *Caluromys* and *Marmosa* lineage(s) is not clearly defined by the available data. D, *Didelphis*; C.A, *Caluromys* and *Marmosa*; T, *Metachirus*; M, Macropodidae; P, Phalangeridae; K.W, Koala and wombat; S, Dasyuridae; B, Peramelidae; W, *H. wrightorum*; Y, *H. chrysoscelis*; C, *H. cinerea*; G, *H. gratiola*; F, *H. femoralis*; A, *Acris crepitans*; U, *H. crucifer*; P, *Pseudacris triseriata*; R, *H. regilla*; L, *Litoria aurea*. A fuller presentation of the hyline phylogeny is made in ref. 29.

which were separated by continental drift, one can test the evolutionary clock hypothesis. For the intercontinental tests described here, we use serum albumin.

Quantitative immunological comparisons indicate that the various albumin lineages have accumulated amino acid substitutions with considerable regularity¹³⁻²⁰, although in birds²⁰ the rate is two or three times lower than in other vertebrates tested. We have been impressed with fragmentary evidence which suggests that the rate of albumin evolution in frogs^{16,18} is similar to that in placental mammals, in spite of the fact that evolution at the organismal level has been slower, by far, in frogs than in mammals²¹⁻²². Because the fossil record of frogs is so inadequate, however, it was difficult to know how similar the rates of albumin evolution have been in the two groups. We therefore sought an alternative approach to the problem of comparing the rates of albumin evolution in organisms with poor fossil records. The problem is of interest not only from the standpoint of trying to understand the mechanisms of protein evolution, but also because albumin and other proteins may prove to be useful evolutionary dating devices.

Recent studies of continental drift provide an appropriate zoogeographical setting to test the albumin clock hypothesis. We have considered the mammal superorder Marsupialia and the tree frog subfamily Hylinae, both of which have a distribution which includes the New World and Australia and excludes Africa and South-east Asia. Studies of global tectonics indicate that South America and Australia were relatively recently connected by way of Antarctica²³⁻²⁵. Thus if the marsupials and tree frogs have a southern continent ancestry, the most recent existence of those proposed ancestors must predate the separation of the two continents. Indeed, disappearance of the trans-Antarctic bridge may have been the event which separated the marsupial and hyline lineages represented today in Australia from those in the New World²⁶.

Albumins were purified from 17 marsupial species (13 Australian, 4 New World) and 10 hylines (9 New World and

Table 1 Immunological comparison of Australian and New World albumins

Taxa compared		Anti-A against B	Immunological distances† Anti-B against A	Mean
Marsupials*				
A	B			
Macropodidae	<i>Caluromys, Marmosa</i>	90	94	92
Macropodidae	<i>Didelphis, Metachirus</i>	115	118	116
Phalangeridae	<i>Caluromys, Marmosa</i>	91	94	92
Phalangeridae	<i>Didelphis, Metachirus</i>	133	131	132
Wombat, Koala	<i>Caluromys, Marmosa</i>	84	84	84
Wombat, Koala	<i>Didelphis, Metachirus</i>	120	117	118
Dasyuridae	<i>Caluromys, Marmosa</i>	82	79	80
Dasyuridae	<i>Didelphis, Metachirus</i>	125	119	122
Peramelidae	<i>Caluromys, Marmosa</i>	80	79	80
Peramelidae	<i>Didelphis, Metachirus</i>	116	119	117
Mean for comparisons involving <i>Caluromys</i> and <i>Marmosa</i>		85	86	86
Mean for comparisons involving <i>Didelphis</i> and <i>Metachirus</i>		122	121	121
Mean for marsupials		103	103	103
Hyelines†				
A	B			
<i>Litoria</i>	<i>Hyla wrightorum</i>	158	113	136
<i>Litoria</i>	<i>H. chrysoscelis</i>	130	103	116
<i>Litoria</i>	<i>H. cinerea</i>	158	146	152
<i>Litoria</i>	<i>H. gratiosa</i>	150	93	122
<i>Litoria</i>	<i>H. femoralis</i>	163	122	142
<i>Litoria</i>	<i>Acris crepitans</i>	105	121	113
<i>Litoria</i>	<i>H. crucifer</i>	141	137	139
<i>Litoria</i>	<i>Pseudacris triseriata</i>	156	102	129
<i>Litoria</i>	<i>H. regilla</i>	129	97	113
Mean for hyelines		144	114	129

*Antisera to the purified albumins of two or more Australian genera in each group and of the four New World genera (*Didelphis*, *Caluromys*, *Marmosa*, *Metachirus*) were used to measure the various immunological distances between the albumins of the respective Australian groups and those of the New World forms. Macropodidae included *Bettongia*, *Macropus*, and *Dendrolagus*; Phalangeridae included *Phalanger*, *Petaurus*, and *Pseudocheirus*; Dasyuridae included *Dasyurus*, *Dasyuroidea*, and *Myrmecobius*; and the Peramelidae were *Echymipera* and *Isoodon*.

†Antisera to the purified albumins of the Australian *Litoria aurea* and all the New World species listed were used in these tests. A fuller presentation of the results of these tests is given in ref. 29.

‡For each pair of species or groups of species two values are given because reciprocal tests were made (that is, anti-A with B and anti-B with A); the last column gives the average of the two.

1 Australian). Antisera to each albumin were prepared by immunising a group of three to five rabbits for 3 months. The methods of purification and immunisation are described elsewhere^{17,18,27}. The antisera to a given albumin were then pooled in inverse proportion to their micro-complement fixation (MCF) titres, and antigenic differences among the various albumins measured by the MCF procedure using these pools²⁸. The degree of antigenic difference between any two albumins is expressed as an immunological distance²⁸ where, for albumin, one immunological distance unit is equivalent to about one amino acid substitution²⁷.

Table 1 shows that the mean immunological distances between the Australian and New World albumins are quite similar, 103 for marsupials and 129 for tree frogs. As the distances range from 80 to 152, however, it seems that albumin evolution has been quite irregular in both the marsupials and hyelines. Thus evolutionary change in the antigenic properties of the albumin molecule in one hyline pair may have developed at almost twice the rate of one marsupial pair. Although the presence of such discrepancies precludes any naive use of an albumin clock, we think that the divergence events at issue can be dated provided the data are properly analysed.

The basic precaution should be to separate out the non-random discrepancies from the random variations which are a component of any non-deterministic clock. The first of these is evident in the marsupial data where the mean immunological distance from the Australian species to *Didelphis* and *Metachirus* is 121 units, whereas that to *Caluromys* and *Marmosa* is only 86 units. The significance of this result does not become evident until a phylogenetic (cladistic) analysis is carried out using all possible intra-Australian and intra-American comparisons in addition to

the intercontinental distances given in Table 1. The results of this analysis are shown in Fig. 1 and indicate first that those species studied on a given continent form monophyletic groups relative to those on the other. The marsupial analysis is facilitated because we are able to use placental mammal albumins as outside reference points to estimate the relative amounts of change along the various marsupial albumin lineages; that is, we assume that the living marsupials share common ancestry subsequent to the divergence of the ancestral placental lineage. As Fig. 1 shows, the lineage(s) leading to *Caluromys* and *Marmosa* has experienced much less albumin change than those of the other marsupials, for whom the amount of albumin change since the separation of the Australian and New World groups is about 61 ± 5 units. Note that the existence of an albumin clock cannot be assumed; it must be documented for the particular group being studied. Thus, times of divergence are calculated by first allocating the amount of albumin change along the various lineages involved, noting if these are similar enough to be compatible with the clock model, and then using the mean amount of change along those lineages consistent with the clock model as a measure of their age. Thus the marked deceleration in albumin change for *Caluromys* and *Marmosa* is clearly indicated by internal analysis and is ignored in dating the separation of the two marsupial groups.

The hyline data illustrate a problem specifically associated with the immunological approach. The anti-*Litoria* values in Table 1 are generally higher than those obtained in reciprocal tests. This is not a unique occurrence as similar degrees of variations exist for a small percentage of the hundreds of antisera prepared in this laboratory. Thus, just as the mean immunological distance between albumins of the Australian and New World marsupials underestimates

the actual phyletic distance (because of the lack of change along the *Caluromys* and *Marmosa* lineages), so too is the corresponding hyline distance perhaps overestimated by giving the anti-*Litoria* values (mean of 144) a weight equal to the nine New World antisera combined (mean 114). To check this, antisera to additional Australian hyline albumins are needed, but until these are prepared it is probably best to accept 114 units as the best available estimate of the intercontinental hyline distance.

The rate constant for albumin evolution in placental mammals and iguanid lizards is about 1.7 units per Myr per pair of lineages compared^{13-15, 17, 18, 30}. If this holds for marsupials and hylines, then for the separation of the Australian and New World faunas, one obtains a figure of 73 Myr for marsupials and 68 Myr for hylines. These figures are consistent with current geological dating of the South America-Antarctica separation as occurring in the late Cretaceous 70 Myr ago²³⁻²⁵.

Thus the sources of our discrepancies have been identified by internal analysis. For the resulting level of accord to exist, the albumins of two groups differing enormously in their histories, ways of life, and rates of morphological evolution must have accumulated approximately equal amounts of immunological change over 70 Myr. These differing rates of morphological evolution are reflected in the formal taxonomies of the groups—all the frogs studied being in the subfamily Hyalinae, whereas it takes a superorder to encompass the marsupials. Indeed, until 1971 *Litoria* was included in the genus *Hyla*³¹. Secondly, for the immunological approach to produce such consistent results, it must be measuring something which is evolving in a regular fashion. This something is presumably surface amino acid substitutions in the several albumin lineages being studied. Direct evidence from immunological studies of proteins of known sequence and configuration indicates that this is the case³²⁻³⁴. Thirdly, the calibration of the albumin clock at 100 units of immunological distance accumulated per 60 Myr of separation provides dates which agree with the latest global tectonics evidence and with vital zoogeographical considerations. If South America and Australia had been connected during the early Tertiary, then one would expect to find evidence of ancient placental lineages in Australia—there is none. On the other hand, if the trans-Antarctic connection had ceased to exist much earlier in time than the late Cretaceous, then one could not have had the appropriate marsupial ancestors in South America in time to get them over to Australia. Finally, the congruence of the marsupial and hyline tree frog pictures discussed here provides in itself strong support for the proposed trans-Antarctic passage of the two groups to Australia—a matter still in some dispute for at least the marsupials³⁵.

Although the disappearance of the trans-Antarctic connection was probably the event which separated the marsupial and hyline lineages existing today in Australia from those in the New World, an alternative should be considered. One or both of the divergences may, in theory, have occurred well before the continents drifted apart. A more ancient divergence coupled with slower albumin evolution would be compatible with the results given above. This alternative is unlikely, however, in view of the phylogenetic position of marsupials within the class Mammalia and of the hylines within the superfamily Bufonoidea. The mean albumin immunological distance between marsupial and placental mammals is about 200 units, which corresponds to an early Cretaceous divergence, in agreement with palaeontological opinion^{36, 37} as well as with sequence evidence derived from haemoglobin and myoglobin⁷. These considerations also suggest that deceleration in albumin change occurs in *Caluromys* and *Marmosa*, rather than acceleration in the other marsupial albumin

lineages studied. Similarly, our intra-bufonoid studies²⁹ indicate maximum albumin immunological distances of about 180 units within that group, which is consistent with other evidence^{38, 39} that the bufonoid frogs arose in South America shortly after its separation from Africa, that is, in the middle Cretaceous period. Thus, consideration of the evolution of hylines and marsupials within a larger taxonomic context does not suggest that albumin evolution has been significantly retarded in marsupials or hylines. We infer, therefore, that the lineages leading to living Australian marsupials and hylines diverged from those leading to their New World counterparts at a time that did not significantly predate the disappearance of the trans-Antarctic land bridge between these two regions.

Even though the albumin clock may not always keep perfect time, it retains considerable potential for evolutionary dating. For that potential to be fully realised, the errors to which the clock is prone must be recognised and, as far as possible, be corrected for. Those which stem from the fact that the albumin clock, to the extent it exists, must be a statistical clock can be minimised by examining as many lineages as possible stemming from a particular divergence point and carrying out parallel studies with additional proteins and nucleic acids where each molecule will provide an independent estimate of the particular divergence time. We have also shown that a phylogenetic (cladistic) analysis of the immunological distance data facilitates the isolation of non-random errors stemming from discordant rates of change and immunological imperfections. We anticipate that such efforts will eventually provide molecular approaches to times of divergence with a resolving power comparable to that available from radiometric dating of objects.

We thank the following who supplied materials used: J. P. Bogart, E. D. Brodie, H. G. Cogger, G. Gentry, J. E. Juterbock, R. D. Maxson, R. C. Richmond, M. Robinson, D. L. Stephan and R. N. Wilson for frogs; R. D. Barnes, J. A. W. Kirsch, E. P. Finnie, W. Mottram, B. J. Richardson and R. B. Tesh for marsupials. We also thank Anne Hill for assistance, the National Science Foundation for financial support to V.M.S. and A.C.W., and the Alpha Helix expedition to New Guinea in 1969 for initiating the marsupial project.

LINDA R. MAXSON
VINCENT M. SARICH
ALLAN C. WILSON

*Departments of Biochemistry and Anthropology,
University of California,
Berkeley, California 94720*

Received July 15, 1974; accepted April 15, 1975.

- Zuckerkandl, E., and Pauling, L., in *Horizons in Biochemistry* (edit. by Kasha, M., and Pullman, B.), 189-225 (Academic, New York, 1962).
- Margoliash, E., *Proc. natn. Acad. Sci. U.S.A.*, **50**, 672-679 (1963).
- Sarich, V. M., and Wilson, A. C., *Proc. natn. Acad. Sci. U.S.A.*, **58**, 142-148 (1967).
- Wilson, A. C., and Sarich, V. M., *Proc. natn. Acad. Sci. U.S.A.*, **63**, 1088-1093 (1969).
- Kohne, D. E., *Q. Rev. Biophys.*, **3**, 327-375 (1970).
- Dickerson, R. E., *J. molec. Evol.*, **1**, 26-45 (1971).
- Air, G. M., Thompson, E. O. P., Richardson, B. J., and Sharman, G. B., *Nature*, **229**, 391-394 (1971).
- Mao, S. H., and Dessauer, H. C., *Comp. Biochem. Physiol.*, **40A**, 669-680 (1971).
- Romero-Herrera, A. E., Lehmann, H., Joysey, K. A., and Friday, A. E., *Nature*, **246**, 389-395 (1973).
- Langley, C. H., and Fitch, W. M., *J. molec. Evol.*, **3**, 161-178 (1974).
- Van Valen, L., *J. molec. Evol.*, **3**, 89-101 (1974).
- Kimura, M., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 2848-2852 (1974).
- Sarich, V. M., *Syst. Zool.*, **18**, 286-295; 416-422 (1969).
- Sarich, V. M., in *Old World Monkeys* (edit. by Napier, J. R., and Napier, P. H.), 175-226 (Academic, New York, 1970).
- Gorman, G. C., Wilson, A. C., and Nakanishi, M., *Syst. Zool.*, **20**, 167-185 (1971).
- Wallace, D. G., Maxson, L. R., and Wilson, A. C., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 3127-3129 (1971).
- Sarich, V. M., *Nature*, **245**, 218-220 (1973).
- Wallace, D. G., King, M.-C., and Wilson, A. C., *Syst. Zool.*, **22**, 1-13 (1973).
- Sarich, V. M., and Wilson, A. C., *Science*, **171**, 1144-1147 (1973).
- Prager, E. M., Brush, A. H., Nolan, R. A., Nakanishi, M., and Wilson, A. C., *J. molec. Evol.*, **3**, 243-262 (1974).
- Wilson, A. C., Maxson, L. R., and Sarich, V. M., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 2843-2847 (1974).

- ²² Wilson, A. C., Sarich, V. M., and Maxson, L. R., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 3028–3030 (1974).
- ²³ Elliot, D. H., Colbert, E. H., Breed, W. J., Jensen, J. A., and Powell, J. S., *Science*, **169**, 1197–1201 (1970).
- ²⁴ Dalziel, I. W. D., and Elliott, D. H., *Nature*, **233**, 246–252 (1971).
- ²⁵ Raven, P. H., and Axelrod, D. I., *Science*, **176**, 1372–1386 (1972).
- ²⁶ Cracraft, J., in *Implications of Continental Drift to the Earth Sciences*, **1**, (edit. by Tarling, D. H., and Runcorn, S. K.), 373–393 (Academic, London, 1973).
- ²⁷ Maxson, L. R., and Wilson, A. C., *Science*, **185**, 66–68 (1974).
- ²⁸ Champion, A. B., Prager, E. M., Wachter, D., and Wilson, A. C., in *Biochemical and Immunological Taxonomy of Animals* (edit. by Wright, C. A.), 397–416 (Academic, London, 1974).
- ²⁹ Maxson, L. R., and Wilson, A. C., *Syst. Zool.*, **24**, 1–15 (1975).
- ³⁰ Wallace, D. G., and Wilson, A. C., *J. molec. Evol.*, **2**, 72–86 (1972).
- ³¹ Tyler, M. J., *Univ. Kansas Publ. Mus. Nat. Hist.*, **19**, 319–360 (1971).
- ³² Wilson, A. C., and Prager, E. M., in *Lysozyme* (edit. by Osseman, E. F., Canfield, R. E., and Beychok, S.), 127–141 (Academic, New York, 1974).
- ³³ Reichlin, M., *Adv. Immun.*, **20**, 71–123 (1975).
- ³⁴ Champion, A. B., Soderberg, K. L., Wilson, A. C., and Ambler, R. P., *J. molec. Evol.* (in the press).
- ³⁵ Keast, A., in *Evolution, Mammals, and Southern Continents* (edit. by Keast, A., Erk, F. C., and Glass, B.), 23–87 (State Univ. New York Press, Albany, 1972).
- ³⁶ Lillegraven, J. A., *Univ. Kansas Paleontol. Contr.*, **50**, (1969).
- ³⁷ Clemens, W. A., Jr., in *Early Mammals* (edit. by Kermack, D. M., and Kermack, K. A.), suppl. 2, 165–180 (*Zool. J. Linn. Soc.*, **50**, 1971).
- ³⁸ Estes, R., and Reig, O. A., in *Evolutionary Biology of Anurans* (edit. by Vial, J. L.), 1–63 (Univ. Missouri Press, Columbia, 1973).
- ³⁹ Savage, J. M., in *Evolutionary Biology of Anurans* (edit. by Vial, J. L.), 351–445 (Univ. Missouri Press, Columbia, 1973).

Chemical communication in maternal behaviour of crayfish

I HAVE found that chemical communication is important in the brooding behaviour of the crayfish. Although chemical sex attractants have been described in the American lobster¹ and Pacific crab⁴, this is the first report concerning maternal care of the young.

The female crayfish carries the eggs and subsequently the larvae on her abdominal pleopods until the third larval stage during which the larvae occasionally leave the female to feed, spending progressively less time with her. Within a few days after they moult into the fourth stage, they no longer return to the female⁶. If third stage larvae are removed from a brooding female and then placed in an aquarium containing the same brooding female and other non-brooding adults, they cluster on the former^{2,3}. Non-brooding crayfish will readily feed on larvae.

These and my own observations suggested that the larvae use chemical cues to identify the brooding females, for I found that they could aggregate about the mother in the absence of visual or physical contact with her.

To investigate this further, I used ovigerous females of *Orconectes sanborni* from north-eastern Ohio, *Cambarus virilis* from central Maine and *Procambarus clarkii* from a population maintained in the laboratory. They were held in isolation in aerated unchlorinated water.

I followed two procedures to determine the larval response to the chemical cues released into the water by adults. In one method, filter paper was used to collect the chemical products released by brooding females. The paper was soaked in the tanks of brooding and non-brooding *P. clarkii* adults for at least 24 h. Three 1.3-cm squares of this treated filter paper from tanks containing both brooding and non-brooding adults were

Table 1 Response of *P. clarkii* larvae to soaked filter paper

No. of larvae used	Stimulus from	No. of larvae responding	No. of experiments	χ^2 Score	P
242	Mother and male	152	24	93.09	0.005
280	Mother and female	163	20	85.34	0.005
180	Male and female	61	12	3.08	0.995

Some larvae did not respond, presumably because of lower activity levels or related motivational factors.

offered to between 10 and 12 third stage *P. clarkii* larvae in a 30.5-cm diameter finger bowl. Larval preference for a given adult's chemical product was indicated by the number of larvae that had aggregated on the paper squares from the tanks of different adults 10 min after the larvae were released from confinement.

Table 1 shows that filter paper from *P. clarkii* brooding females was significantly preferred over those from the tanks containing males or females. The larval response to the papers of males and non-brooding females was not significant. This shows that the larvae discriminate by means of chemical factors released into the water by adults. The products released by the mother are different from those of other adults and the larvae show a significant preference for them, indicating the attractive quality of her cue.

My second approach tested the larvae's attraction when water in which brooding and non-brooding adults had been held, was presented in a two-choice maze⁵. In preparing the chemical stimuli the adults had to be held in the stimulus chambers for 45–60 min to evoke a response from the larvae (Fig. 1). After this water conditioning period the adults were removed from the maze and larvae were released. Larval preference was indicated by their movement from the main chamber, where they were released, into the test chambers, which contained the chemical stimulus of either brooding females or non-brooding adults.

To prevent the adults' stimuli from mixing in the test chambers, 200 ml of fresh water was added to the stimulus chambers at a rate of 150 ml min⁻¹ every 15 min. The larvae that had moved into each test chamber were counted before and 3 min after each rinse. After three or four rinses, the adults were again placed in the maze, but this time the position of the adults in the stimulus chamber was switched, to control for other stimuli which might bias the preference of the larvae. After a 60-min

Fig. 1 Two-choice maze. Larvae responded to the stimuli of the adults by moving from the main chamber into the test chamber containing water in which either a brooding female or male had been held.

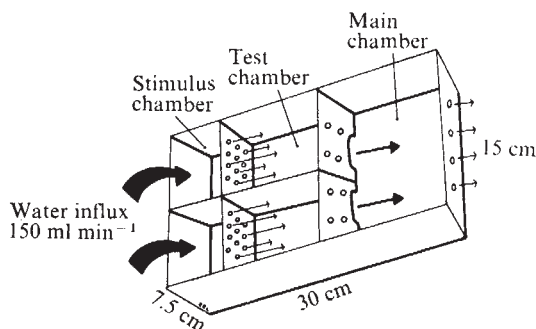


Table 2 Larval response in the maze

Species	No. of larvae used	Stimulus from	No. of larvae responding	No. of experiments	χ^2 Score	P
<i>P. clarkii</i>	375	Mother and male	242	18	124.51	0.005
<i>O. sanborni</i>	527	Mother and male	310	24	135.64	0.005
<i>C. virilis</i>	180	Mother and male	101	4	23.36	0.005

The results shown are the sum of the larval count from each experiment in which the greatest total number of larvae responded to both adult stimuli. As in Table 1, some larvae made no response.

water conditioning period, the adults were removed from the maze and larvae were counted as before.

Brooding females were always stripped of larvae before being used as maze stimuli, to control for possible larval stimuli, and conspecific non-brooding adults served as controls since their stimulus was not attractive.

In this manner 1-d-old third stage larvae of all three species were given a choice between the chemical stimuli of a brooding female and a male. In all experiments, the larvae of each species showed a significant preference for the brooding female (Table 2), by consistently selecting her side of the maze, even after her position in the maze had been changed.

The results of these experiments demonstrate that larval discrimination is based on a chemical stimulus released into the water by the brooding females. These cues must be important for the survival of the larvae in that they attract them to the female, thus facilitating the protective functions of the brooding interaction. Limited observation suggests that the chemical cues are species specific. They are not brood specific, however, for larvae are attracted as strongly to the other conspecific brooding females as to their own mother.

The research was supported by a grant from the US National Science Foundation. I thank A. Carlson, R. Hoy, D. Smith and C. Walcott for help.

EDWARD E. LITTLE*

Division of Biological Sciences,
State University of New York,
Stony Brook, New York 11790

Received February 25, 1974; accepted April 17, 1975.

*Present address: Department of Biological Sciences, Florida State University, Tallahassee, Florida 32306

1 Atema, J., and Engstrom, D. G., *Nature*, **232**, 261-263 (1972).

2 Chidester, F., *Am. Nat.*, **46**, 279-293 (1912).

3 Pieplow, U., *Z. Fisherie. Hilfswiss.*, **36**, 350-437 (1938).

4 Ryan, E., *Science*, **151**, 340-341 (1966).

5 Shelton, R., and Mackie, A., *J. exp. Biol. Ecol.*, **7**, 41-49 (1971).

6 Tack, P., *Am. Mid. Nat.*, **25**, 420-444 (1941).

Non-Mendelian streptomycin-resistant tobacco mutant with altered chloroplasts and mitochondria

THE possibility of producing mutant plants of economic value by tissue culture techniques has attracted considerable interest and discussion¹⁻⁶, and was confirmed by the regeneration of plants from mutant cell lines¹. The isolation of cytoplasmic mutants (all of those studied so far have carried Mendelian traits^{1,2}) can be of practical importance; for example, male sterile ones in maize may be used for producing hybrid seeds. We report here, however, properties of a non-Mendelian, cytoplasmic mutant.

Streptomycin-resistant *Nicotiana tabacum* mutant SR1 (previously referred to as Str-r₁) was one of the first mutants isolated *in vitro* from cultured plant tissue and grown to a flowering plant⁷. A detailed study of the progeny of SR1 plants (F₁, F₂ and backcrosses) indicated that resistance is determined by a non-Mendelian, cytoplasmic factor. Ultrastructural changes of chloroplasts and mitochondria induced by streptomycin in streptomycin-sensitive (SS) cells were absent in the SR1 material when grown *in vitro* on a drug-containing medium, suggesting that mutation affected the organelles.

To test the progeny of SR1 plants, surface-sterilised seeds from selfing and crosses were germinated on water-agar and 10-d-old seedlings inoculated on to RMO medium (see ref. 7) with and without 5 and 500 µg ml⁻¹ streptomycin, respectively. After 4 weeks, fresh weight of callus obtained from the seedlings was determined. Data from progeny tests are shown in Fig. 1.

SR1 seedlings from selfing, and those obtained by pollinating SR1 plants with pollen from SS plants (SR1 × SS cross), gave rise to streptomycin-resistant calli (that is, the growth of these calli was much less inhibited by streptomycin than that of the

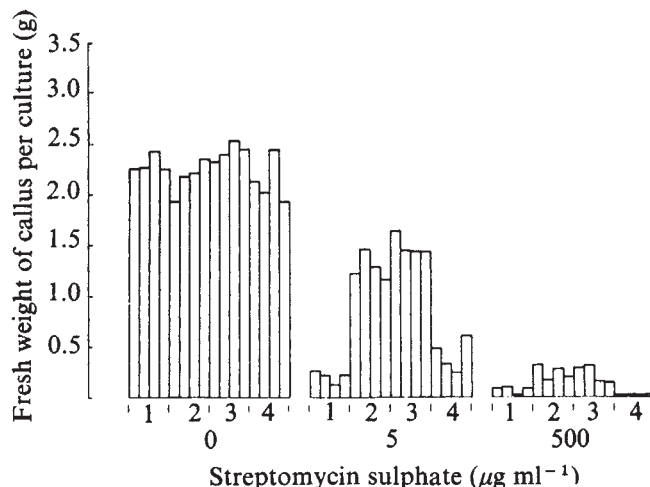


Fig. 1 Transmission of streptomycin-resistance in F₁, F₂ and backcrosses with plants regenerated from the SR1 cell line. For the test, seedlings (10 mg) were inoculated on to an RMO medium⁷ (5 ml) in penicillin flasks containing different amounts of streptomycin. Seeds were obtained by crosses pollinating SS, SR1, SR1 × SS, and SS × SR1 plants (in groups from one to four, respectively) with pollen from SS, SR1, SR1 × SS, and SS × SR1 plants (columns from left to right within each group). Fresh weights (averages of ten cultures) were determined after a 4 week growth period. S.e. about 10% in each case.

SS ones). SS and SS × SR1 seedlings were drug-sensitive when tested as calli on streptomycin medium. The behaviour of calli of seedlings from F₂ and backcrosses of F₁ to SR1 and SS was also always determined by the maternal phenotype. This indicates that streptomycin resistance was transmitted in a non-Mendelian, uniparental way, as other cytoplasmic genes of tobacco⁸.

Mutation in the SR1 cell line for streptomycin resistance was completely stable. So far, not one sensitive segregant was found among 100 seedlings tested from the selfed material, and no genetic change other than mutation for streptomycin resistance was observed. The diploid SR1 plants growing in a greenhouse in the absence of streptomycin could not be distinguished morphologically from the SS ones and those obtained from crosses.

In higher plants streptomycin is known to exert its toxic effect by inhibiting protein synthesis on bacterial-type ribosomes of chloroplasts and mitochondria⁹. Resistance to streptomycin of SR1 chloroplasts and mitochondria was directly confirmed by electron microscopy.

Small leaves were taken from shoots of callusing SR1 and SS seedlings, grown for 5 weeks on RMO medium, with and without 500 µg streptomycin ml⁻¹. (Shoots of SS seedlings on the drug were rudimentary.) Leaf cubes were then dissected, fixed in aldehyde solution of high osmolarity¹⁰, treated with osmium tetroxide and embedded in araldite¹¹. Thin sections of silver interference colours were viewed in a Jeol 100B electron microscope.

Ultrastructural changes of chloroplasts and mitochondria, known to occur in the SS material after streptomycin treatment¹², were found in the SS cells grown in the presence of the drug. Membranes and grana were hardly recognisable in the chloroplasts and an increase in electron density of the mitochondria was frequently found. In the SR1 cells, however, grana and stroma lamellae developed in the chloroplasts even in the presence of streptomycin. The grana were ill-defined and consisted of two to three thylakoids only, possibly as a result of heavy drug treatment. The fine structure of mitochondria seemed to remain intact.

Resistance of SR1 chloroplasts and mitochondria themselves is not the only explanation for the unimpaired ultrastructure of the organelles on a streptomycin medium. Mutation, affecting penetration of the drug into the cells or its inactivation by chemical modification (as is the case with certain bacterial

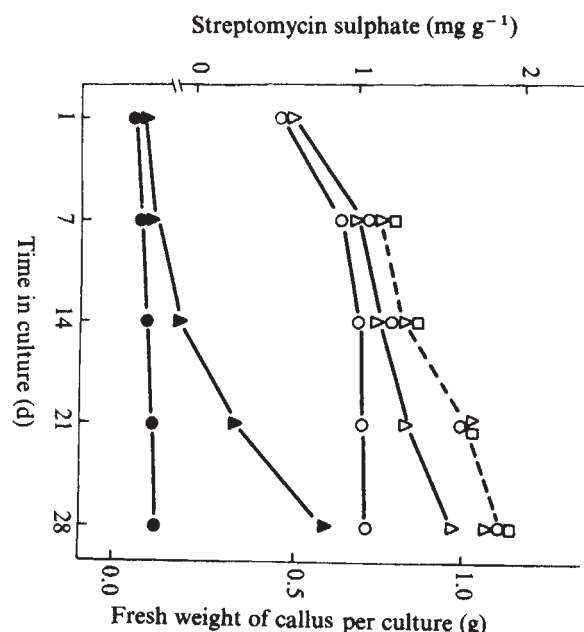


Fig. 2 Streptomycin concentration in SR1 and SS calli, and in their culture media during a 4 week growth period. Calli were grown on a medium containing 1 mg ml⁻¹ streptomycin sulphate, as described in Fig. 1. Δ and $\bullet$, average fresh weight of ten SR1 and SS callus cultures; solid line, streptomycin concentrations in the calli for SR1 (Δ) and SS ($\bullet$); broken line, concentration of streptomycin in culture media for SR1 (Δ) SS ($\bullet$) and uninoculated cultures ($\square$).

strains¹³), could equally protect organelles. To exclude these latter possibilities, we studied the uptake of streptomycin in callus culture. SR1 and SS calli, grown on a streptomycin medium, were collected at weekly intervals and their fresh weights, the amount of streptomycin per unit fresh weight in calli, and culture medium, respectively, were determined (Fig. 2).

Streptomycin was estimated in the supernatant of calli homogenised in water, and in water extracts of the culture media by the photometric method of Duda¹⁴, except that the reaction mixture was incubated at 25 °C. The test is highly specific for streptomycin; none of the compounds present in a callus water extract or decomposition products of streptomycin, as well as other aminoglycoside antibiotics, such as neomycin, kanamycin, gentamycin, interfere (L. M., G. Kiss and E. Duda, unpublished). Agar-gel diffusion assay of the same extracts was also performed with *Bacillus subtilis* and drug concentrations were calculated from the size of inhibitory zones. As data obtained by bioassay and chemical determination were quantitatively in good agreement only one value is given in Fig. 2.

On RMO medium containing 1 mg ml⁻¹ streptomycin sulphate only SR1 inocula proliferated, although there was no difference in growth in the absence of the drug (Fig. 1). Approximately the same drug concentrations were found in both SR1 and SS calli and also in their culture media, and no difference in drug content of inoculated and uninoculated media was found. Similar results were obtained when applying the drug in different amounts (not shown). Increase in drug concentration of the medium was caused by evaporation.

It is very improbable that a secondary product of streptomycin breakdown kills *B. subtilis* but still leaves the SR1 cells unharmed, because that particular derivative ought to give quantitatively the same reaction and the same activity in bioassay as streptomycin. The concentration chosen (1 mg ml⁻¹) was selected because of the convenience of quantification. When inoculating cultures with callus a much higher drug concentration was necessary to obtain difference in growth between SR1 and SS material compared with those inoculated with seedlings (Fig. 1 and Fig. 1 of ref. 7).

Uptake experiments suggest that, as in non-Mendelian

streptomycin-resistant mutants of the green alga, *Chlamydomonas*¹⁵, cytoplasmic mutation in SR1 cells affects the organelles as streptomycin was neither excluded from the SR1 cells as a result of a change in membrane permeability nor inactivated within the SR1 cells or outside in the medium.

An important inference from the present study, regarding practical applications, is that growth of cells in tissue culture under selective pressure does not necessarily induce any heritable alteration beyond the desired mutation; and, although streptomycin-resistance was obtained in cultured somatic cells, the factor responsible was transmitted by the germ cells to the progeny of the regenerated plants, and behaved as any other cytoplasmic factor detected in and described for whole tobacco plants.

We thank Drs G. Kiss and E. Duda of BRC, Szeged, for communicating their unpublished results.

P. MALIGA
AGNES SZ.-BREZNOVITS
L. MARTON

Institute of Plant Physiology,

F. JOO

Institute of Biophysics,
Biological Research Centre,
Hungarian Academy of Sciences,
6701 Szeged, POB 521, Hungary.

Received December 9, 1974; accepted April 17, 1975.

- Carlson, P. S., Dearing, R. D., and Floyd, B. M., in *Genes, Enzymes and Populations* (edit. by Srb, A. M.), 99–107 (Plenum, New York and London, 1973).
- Chaleff, R. S., and Carlson, P. S., *A. Rev. Genet.*, **8**, 267–278 (1974).
- Melchers, G., in *Protoplastes et fusion de Cellules Somatiques Végétales* (edit. by Tempé, J.), 367–372 (CNRS, Paris, 1973).
- Nickell, L. G., and Heinz, D. J., in *Genes, Enzymes and Populations* (edit. by Srb, A. M.), 109–128 (Plenum, New York and London, 1973).
- Smith, H. H., *Bioscience*, **24**, 269–276 (1974).
- Widholm, J., *Plant Sci. Lett.*, **3**, 323–330 (1974).
- Maliga, P., Sz-Breznovits, A., and Márton, L., *Nature new Biol.*, **244**, 29–30 (1973).
- Smith, H. H., *Adv. Genet.*, **14**, 1–54 (1968).
- Pestka, S., *A. Rev. Microbiol.*, **25**, 487–562 (1971).
- Karnovsky, M. J., *J. Cell Biol.*, **27**, 137A–138A (1965).
- Millonig, G., *J. appl. Phys.*, **32**, 1637 (1961).
- Behn, W., and Arnold, C. G., *Protoplasma*, **82**, 77–89 (1974).
- Benveniste, R., and Davies, J., *A. Rev. Biochem.*, **42**, 471–506 (1973).
- Duda, E., *Analyt. Biochem.*, **51**, 651–653 (1973).
- Gillham, N. W., *A. Rev. Genet.*, **8**, 347–391 (1974).

Inhibition by parasites of melanotic tumour formation in *Drosophila melanogaster*

THE cellular defence mechanisms of insects are mediated by blood cells, or haemocytes, which eliminate invading foreign organisms by phagocytosis and encapsulation. The latter involves aggregation, adhesion and flattening of haemocytes around surfaces too large to be engulfed. This process is characteristically accompanied by the intra- and extracellular deposition of melanin^{1–5}. In larvae of *Drosophila* the reaction against internal metazoan parasites^{6–8} is similar to the reaction which forms melanotic tumours in the body cavity of some mutant strains^{9–14}. In both cases haemocytes proliferate and differentiate prematurely. In some tumorous larvae the formation of these benign, inheritable masses represents an autoimmune response in which haemocytes encapsulate abnormal cells and tissues and form compact melanised masses¹¹.

I report here the complete absence of the tumour phenotype when larvae of the mutant strain *vg tu²⁸* are parasitised by the endoparasitic wasp, *Pseudeucoila bochei*. The female *P. bochei* attacks the larval stages of various species of *Drosophila*, ovipositing a single egg within the body cavity of each. When developed, the adult chews a hole through the puparium of the host and emerges. I investigated whether the initial haemocytic reactions of tumorous larvae against abnormally developing host tissues would also encapsulate the egg of the parasite, or if the normally successful parasite could not only suppress the cellular immune reaction, but also inhibit the autoimmune response associated with tumorigenesis. The only previous

Table 1 Effect of parasitisation by *P. bochei* on the incidence of tumours in *vg tu²⁸*

No. of pupae	% Tumours	% Encapsulated parasites
Non-parasitised pupae		
118	44.91	
61	47.54	
107	50.46	
27	51.85	
88	59.09	
69	39.13	
	Mean 48.80	
Parasitised hosts containing live parasites		
210	0.0	0.0
Parasitised hosts containing dead parasites		
7	57.1	0.0

attempt to parasitise a tumorous insect to observe altered cellular responses was made by Walker⁶ who used resistant and susceptible strains of *P. bochei*.

I examined the effects of parasitisation on tumorigenesis by parasitising *vg tu²⁸* larvae during the early second instar (72 ± 4 h), about 24 h before tumours usually appeared. The larvae were exposed to the parasites for 2 h. Tests were conducted in a regimen of 16 h light at $23 \pm 1^\circ\text{C}$. All hosts were dissected 2–4 d after pupation to determine the condition of the parasite and the effect of parasitisation on the cellular reactions associated with encapsulation and tumorigenesis.

In non-parasitised larvae the incidence of melanotic tumours in *vg tu²⁸* was approximately 48% (Table 1). Most larvae had one large tumour, usually in the posterior region of the body cavity. Occasionally there were four or five large pigmented masses (Fig. 2). No attempt was made to classify separately individuals with either multiple tumours or tumorous masses of different sizes. The tumours comprised fat body cells, encapsulated by haemocytes and melanised (Fig. 2). All tumours were formed before pupation, usually during late second and early third (last) instars. Tumours were retained within the body cavity throughout development and into the adult stage with no evidence of dissolution.

Hosts containing live parasites developed no melanotic tumours. Of the 217 parasitised insects dissected, seven hosts each contained a dead parasite egg. Only four of the seven hosts (57.1%) had the tumour phenotype (Table 1). There was no evidence of parasite encapsulation in any host examined. I do not know why a small percentage of eggs failed to develop, but as this is also encountered in non-tumorous host larvae, female *P. bochei* may sometimes

Fig. 1 Third-instar larva of a tumour strain (*vg tu²⁸*) of *D. melanogaster*. Four large melanotic tumours can be seen through the body wall of the insect. Tumour, Tu; mouth hooks, Mt. The bar represents 0.2 mm.

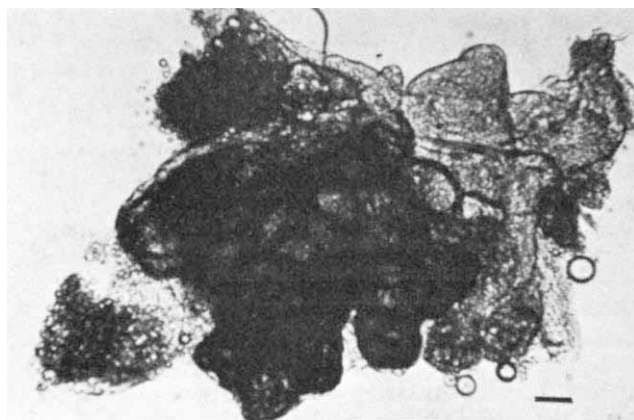
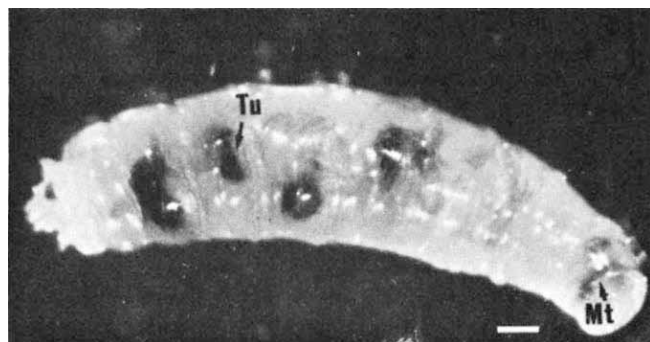


Fig. 2 Melanotic tumour dissected from the body cavity of a third-instar larva. The tumour is composed of abnormal fat body cells in the process of becoming completely melanised and encapsulated. The bar represents 0.02 mm.

oviposit inviable eggs. In a group of infected *vg tu²⁸* pupae that was not dissected, the parasites developed and emerged with no apparent difficulty.

It is interesting that some parasites can develop within hosts that are potentially immunologically hostile. These states of apparent immunological inactivity may represent specific failures of the immune system to recognise parasites as foreign, or they may be attributed to the ability of certain parasites to inhibit host immune responses. Such an encapsulation-inhibiting capacity has been proposed for *P. bochei*^{6,7,15} and other insect parasites^{16,17}. Walker's⁶ studies of the cytological and genetic aspects of capsule formation in *D. melanogaster* suggest that the parasite produces a substance(s) which suppresses the proliferation and differentiation of host haemocytes. In a tumour-mutant stock (*tu B³*) she found that the frequency of tumours was reduced from 71% to about 47% when the larvae were parasitised by *P. bochei* that were normally resistant to encapsulation, and slightly higher (80%) when infected by a parasite strain susceptible to encapsulation.

Regardless of whether the cellular mechanisms of *D. melanogaster* are activated in response to endoparasites or to the asynchronous growth and differentiation of certain host tissues, the haemocytic reactions are similar and serve to isolate and prevent the establishment of foreign or abnormal cells. The results reported here and earlier^{6,7,15} suggest that the ability of *P. bochei* to suppress the encapsulation reaction of its host also inhibits a similar haemocytic response associated with tumorigenesis. Resistance of *P. bochei* in larvae of *Drosophila* may be associated with a substance elaborated from the egg of the parasite, for only in hosts containing viable *P. bochei* eggs were tumour encapsulation reactions blocked. Other factors, however, including compatible surface properties may also be involved in the resistance of *P. bochei*, for dead eggs were not encapsulated, even in hosts which formed tumours.

I thank Dr Burke Judd of the Department of Zoology and the Genetics Foundation at the University of Texas at Austin for the tumour strain. This work was supported by the Research Foundation of the State University of New York and the National Institutes of Health.

A. J. NAPPI

Biological Sciences,
State University of New York,
Oswego, New York 13126

Received March 3; revised April 10, 1975.

¹ Salt, G., *The Cellular Defence Reactions of Insects* (Cambridge University Press, Cambridge, 1970).

- ² Poinar, G. O., Jr, in *Immunity to Parasitic Animals* (edit. by Jackson, G. J., Herman, R. A., and Singer, I.) (Appleton-Century-Crofts, New York, 1969).
³ Whitcomb, R. F., Shapiro, M., and Granados, R. R., in *Physiology of Insecta* (edit. by Rockstein, M.), 5 (Academic, New York, 1974).
⁴ Nappi, A. J., in *Contemporary Topics in Immunobiology* (edit. by Cooper, E. L.) (Plenum, New York, 1974).
⁵ Nappi, A. J., in *Immunity to Animal Parasites* (edit. by Maramorosch, K., and Shope, R.) (Academic, in the press).
⁶ Walker, I., *Rev. Suisse Zool.*, 68, 569-632 (1959).
⁷ Nappi, A. J., and Streams, F. A., *J. Insect Physiol.*, 15, 1551-1566 (1969).
⁸ Nappi, A. J., *J. Parasitol.* (in the press).
⁹ Oftedal, P., *Z. Indukt. Abst. Vererbungslehre*, 85, 408-422 (1953).
¹⁰ Castiglioni, M. C., *Ricerca. scient. Suppl.*, 27, 51 (1957).
¹¹ Rizki, T. M., *J. Morph.*, 100, 459-472 (1957).
¹² Perotti, M. E., and Bairati, A., Jr, *J. Invertebr. Pathol.*, 10, 122-138 (1968).
¹³ Rizki, T. M., and Rizki, R. M., *J. Invertebr. Pathol.*, 24, 37-40 (1974).
¹⁴ Rizki, T. M., and Rizki, R. M., *Experientia*, 30, 543-546 (1974).
¹⁵ Streams, F. A., and Greenberg, L., *J. Invertebr. Pathol.*, 13, 371-377 (1969).
¹⁶ Schneider, F., *Vjschr. Naturf. Ges. Zuerich*, 95, 22-44 (1950).
¹⁷ Kitano, H., *J. Insect Physiol.*, 20, 315-327 (1974).

Antibody stimulation of tumour growth in T-cell depleted mice

It is well known that in certain conditions the growth of allogeneic tumours can be enhanced by passively transferred antibodies directed against antigens present on the graft^{1,2}. Most of the hypotheses proposed to explain this phenomenon have placed some form of antibody-mediated interference with the immune response of the host towards the tumour. Nevertheless, there have been occasional observations which suggest that immunological enhancement is the result of a change in the neoplastic cells themselves resulting from the interaction with antibody³⁻⁵. We have reported the results of *in vitro* experiments which support this idea⁶. Nucleoside incorporation, DNA synthesis, and cell growth were stimulated when mouse L cells were incubated in the presence of limiting amounts of rabbit anti-L cell serum (aLS); higher concentrations of antibody inhibited cell growth. Addition of complement greatly augmented cell growth, especially at low concentra-

tions of antibody⁷. We now report that this immunostimulation phenomenon can also be observed *in vivo*. A preliminary report of this work has appeared⁸.

To provide evidence that the accelerated tumour growth was not the result of an antibody-mediated alteration in the competence of the host to reject the tumour, animals with severely compromised cellular immune functions were used. The hosts were thymectomised C3H/He mice which had received 850 rad of γ -radiation and an intravenous injection of $5-10 \times 10^6$ syngeneic bone marrow cells one day before inoculation with tumour cells. It has been previously shown that thymectomised, irradiated, bone marrow reconstituted (Tx-B) mice have greatly impaired cellular immunity^{9,10}. That this was true for the mice used in our experiments was verified by measuring the negative proliferative response of their spleen cells to the T-cell motigen, phytohaemagglutinin (PHA) according to the method of Stobo and Paul¹¹ (data not shown).

The tumour used in these studies was the mouse fibroblast line, L-929 (Grand Island Biological). This cell line was chosen because it has been previously shown to undergo immunostimulation *in vitro*⁶. Each animal received 2×10^6 cells suspended 0.1 ml of Eagle's MEM containing 10% heat-inactivated (56 °C, 30 min) foetal calf serum. The inoculations were made subcutaneously into previously shaved sites on the flank. The diameter of the resulting tumour was measured with calipers in two dimensions at right angles. Volume was then calculated by applying the approximation formula $V = 0.4ab^2$ where a is the larger and b the smaller of the two dimensions¹².

The preparation of the aLS used in these experiments has been described previously⁶. Briefly, randomly bred New Zealand white rabbits were immunised on three occasions with 2×10^6 L cells (twice in Freund's adjuvant and once

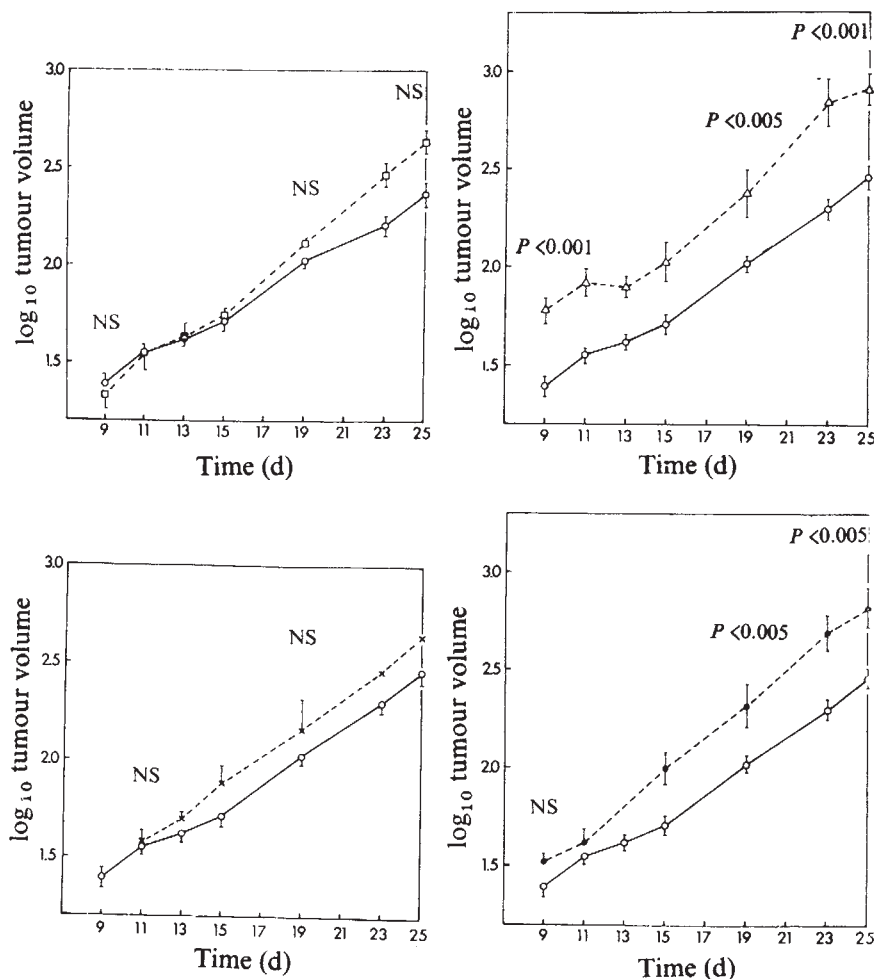


Fig. 1 Subcutaneous growth of L-929 cells in Tx-B C₃H/He mice. Previously thymectomised mice were irradiated, and reconstituted with $5-10 \times 10^6$ syngeneic bone marrow cells on day -1. On day 0, these animals were inoculated with 2×10^6 tumour cells. Group A ($n = 17$; $\circ$) represents pooled data from six experiments in which mice were treated with NRS. Mice in group B (three experiments; $n = 7$; $\square$) received 500 μ l of aLS on day -1. Mice in group C (two experiments; $n = 7$; $\triangle$) received 50 μ l of aLS on day -1. Mice in group D (two experiments; $n = 4$; $\times$) received 5 μ l of aLS on day -1. Mice in group E (two experiments; $n = 7$; $\bullet$) received 50 μ l of aLS on days -1, 5, 10, 15 and 20. Vertical bars represent s.e.m. Day 25 was chosen as the end of the observation period because significant mortality was observed beyond that time in all animals under study. NS, not significant.

in Hank's balanced salt solution). The injections were given at 2-week intervals and the serum was collected four months after the last injection. Normal rabbit serum (NRS) was prepared in the same way except that no cells were present in immunising injections. Sera were heat-inactivated (56 °C, 30 min) and absorbed for 1 h at room temperature with a crude cell suspension of C3H liver, spleen, kidney and heart (10 ml of serum per ml of packed cells).

The design of all the experiments was similar. In each instance, groups of Tx-B mice were given an intraperitoneal injection of NRS or aLS on day -1. The required amount of serum was diluted (if necessary) to 0.5 ml with phosphate buffered saline. On the following day (day 0), the mice were inoculated with tumour cells. In one experiment, additional injections of serum were given subsequent to day 0. Each separate experiment included a control group of animals which were treated identically to the experimental mice, except that NRS was substituted for aLS. Since it was found that tumour growth rate was independent of the dose or schedule of administration of NRS, data from these control mice were pooled (group A). Data obtained from mice which were treated with aLS (groups B, C, D, E) were compared with these control values, and the significance of differences determined by Student's *t* test.

The results of these experiments are depicted in Fig. 1. Animals in group B received 500 µl of aLS on day -1. This quantity of aLS produced a statistically insignificant increase in the rate of tumour growth. Animals in group C received 50 µl of aLS on day -1. Tumour growth was clearly stimulated by this quantity of antiserum (day 9, $P < 0.001$; day 19, $P < 0.005$; day 25, $P < 0.001$). Additional experiments (not shown) showed that at this dose of antiserum, immunostimulation occurred over a twofold range of cell number, that is, 1×10^6 – 2×10^6 cells injected were stimulated by antiserum. When the dose was decreased to 5 µl (group D) this stimulation was no longer seen. Mice in group E received 50 µl of aLS on days -1, 5, 10, 15 and 20. This treatment schedule resulted in an increased rate of tumour growth which was again highly significant (day 9, $P > 0.05$; day 19, $P < 0.005$, day 25, $P < 0.005$.) The experiments did not attempt to answer the question of invasiveness of the tumour. The tumour grew as a solitary nodule with an occasional satellite nodule. The animals' viscera were not examined histologically for tumour metastases.

Two of our findings warrant particular discussion. First, while immunostimulation was observed when the mice were treated with 50 µl of aLS on day -1, a tenfold increase or decrease in dose abrogated the effect. Similar dose-dependent results have been described in previous studies of immunological enhancement^{13,14}. Presumably, antibody is cytotoxic when present *in vivo* at high concentration (as is true *in vitro*). This cytotoxicity would nullify its ability to stimulate cells. Second, in animals given 50 µl of aLS initially, the stimulation was the same regardless of whether the dose was given only once or repeatedly. This can be explained if it is assumed that stimulation is a relatively early event in the development of the tumour. This may be so for several reasons. (1) Growth *in vivo* alters the antigenic characteristics of the tumour cell membrane. This is unlikely because rabbit immunoglobulin is demonstrable by immunofluorescence in tumour sections taken from mice treated with aLS whereas no immunofluorescent staining is present in sections taken from controls treated with NSR. (2) As the tumour develops, there is an increase in the number of antigenic sites and the quantity of antibody bound per cell is no longer sufficient to produce stimulation. (3) Beyond a certain tumour volume, the growth rate of the tumour is limited by the rate of vascularisation and the exchange of metabolites, and stimulation can no longer be detected.

The L cell strain used in these experiments did not produce macroscopic tumours in normal C3H mice regardless of whether the mice were completely untreated or given 50 µl of NRS or aLS. In contrast, progressive growth was also observed in Tx-B mice. Along with PHA data, these results emphasise the low level of host anti-tumour immunity in the Tx-B mice. Furthermore, preliminary experiments with congenitally athymic 'nude' mice confirm the results obtained in Tx-B mice. That is, treatment on day -1 with aLS stimulated the growth of L cell tumours. Thus, it is improbable that the observed stimulation of tumour growth was caused by a blocking effect of the rabbit antibody on tumour allograft rejection. Rather, since this same aLS stimulates L cells to grow faster *in vitro* in the absence of lymphocytes or antibodies from the host animal⁶ the key event *in vivo* is likely to be an alteration in the physiology of the tumour cell occurring as a direct consequence of its interaction with antibody. There have been previous reports of lymphocyte mediated immunostimulation¹⁵⁻¹⁷, but to our knowledge, this is the first evidence presented for immunostimulation mediated by antibody in an *in vivo* system. These observations indicate a continuing need to consider immunostimulation as well as immunoblockade in antibody-mediated effects on tumour growth.

We thank Judith Crouch for technical assistance. This work was supported by a grant from the National Institutes of Health, a Research Scholar Award of the Cystic Fibrosis Foundation to W.T.S., and by a grant from the American Cancer Society.

MITCHELL P. FINK
CHARLES W. PARKER

Department of Medicine

WILLIAM T. SHEARER

Department of Pediatrics,
Washington University School of Medicine,
St Louis, Missouri 63110

Received February 17; accepted April 2, 1975.

- ¹ Voisin, G. A., *Prog. Allergy*, **15**, 328-485 (1971).
- ² Feldman, J. D., *Adv. Immun.*, **15**, 167-214 (1973).
- ³ Gorer, P. A., *Ann. N. Y. Acad. Sci.*, **73**, 707-721 (1958).
- ⁴ Amos, D. B., and Wakefield, J. D., *J. natn. Cancer Inst.*, **22**, 1077-1092 (1959).
- ⁵ Amos, D. B., Prioleau, W. H., and Hutchin, P., *J. surg. Res.*, **8**, 122-127 (1968).
- ⁶ Shearer, W. T., Philpott, G. W., and Parker, C. W., *Science*, **182**, 1357-1359 (1973); *J. exp. Med.*, **139**, 367-379 (1974); *Cell. Immun.* (in press).
- ⁷ Shearer, W. T., Atkinson, J. P., Frank, M. M., and Parker, C. W., *Clin. Res.*, **22**, 612A (1974).
- ⁸ Fink, M. P., Parker, C. W., and Shearer, W. T., *Clin. Res.*, **22**, 610A (1974).
- ⁹ Miller, J. F. A. P., Doak, S. M. A., and Cross, A. M., *Proc. Soc. exp. Biol. Med.*, **112**, 785-792 (1963).
- ¹⁰ Aisenberg, A. C., *J. exp. Med.*, **131**, 275-286 (1970).
- ¹¹ Stobo, J. D., and Paul, W. E., *Cell. Immun.*, **4**, 367-380 (1972).
- ¹² Attia, M. A., Deome, K. B., and Weiss, D. W., *Cancer Res.*, **25**, 451-457 (1965).
- ¹³ Boyse, E. A., Old, L. J., and Stockert, E., *Nature*, **194**, 1142-1144 (1962).
- ¹⁴ Hutchin, P., Amos, D. B., and Prioleau, W. H., *Transplantation*, **5**, 68-75 (1967).
- ¹⁵ Prehn, R. T., *Science*, **173**, 170-171 (1973).
- ¹⁶ Umiel, T., and Trainin, N., *Transplantation*, **18**, 244-250 (1974).
- ¹⁷ Carnaud, C., Ilfeld, D., Levo, Y., and Trainin, N., *Int. J. Cancer*, **14**, 168-174 (1974).

Killer T cells in a Burkitt's lymphoma biopsy

ONE of the *in vitro* tests available for analysis of the interaction between a tumour and its host measures the ability of isolated lymphoid cells to kill tumour cells¹. This killing is believed to reflect cell-mediated immunity *in vivo*, which is a property of thymus-derived (T) lymphocytes. For this test lymphoid cells have been used from sites such as blood, spleen, lymph nodes and thymus. But if a cell-mediated immune response is influencing tumour growth, active lymphoid cells should be present at the site of the tumour. Indeed pathologists recognise lymphoid infiltration of tumours as a prognostic sign².

We have reported that patients with infectious mononucleosis (IM) have killer cells, probably T cells, in the peripheral blood, which specifically kill Epstein-Barr virus (EBV)-genome-positive cell lines derived from normal subjects as well as those derived from patients with IM or Burkitt's lymphoma (BL)³. The aetiological role of EBV is

Table 1 Target cell lines

Name	Origin	Characteristics
Oduor	BL ³	B cell markers EBV genome positive
Kaplan PS-B-1	IM ³ Established from normal lymphocytes by <i>in vitro</i> trans- formation with EBV ³	EBV genome positive EBV genome positive
K-562	Chronic myelocytic leukaemia ¹¹	No B or T cell markers Very sensitive to the cytotoxic action of normal lymphocytes ¹⁰

established in IM⁴, and has been suggested in BL and nasopharyngeal carcinoma (NPC)⁵. With few exceptions BL and NPC tumour cells contain the EBV genome^{5,6}. The humoral immune response, against EBV-determined antigens, in patients with IM, BL and NPC has been studied extensively⁵. We have now isolated a T-cell population from an exceptionally large BL biopsy and found that it can kill the autologous tumour cells and EBV positive cell lines.

The BL biopsy was sent to Stockholm from Kenyatta Hospital, Nairobi, and a cell suspension was prepared. This suspension was centrifuged on Ficoll-Isopaque⁷ to remove debris and dead cells. We obtained approximately 3×10^9 cells. About 1% T cells were present in the suspension as estimated by sheep red blood cell (SRBC) rosette formation⁸. These were isolated by a second buoyant density centrifugation of SRBC rosettes on Ficoll-Isopaque⁹. A purity of 50% T cells with a total yield of 3×10^6 lymphocytes was achieved. Peripheral lymphocytes from a case of IM and from a normal EBV-seropositive person were used unfractionated and after removal of cells with complement receptors by buoyant density centrifugation of complement rosettes⁹.

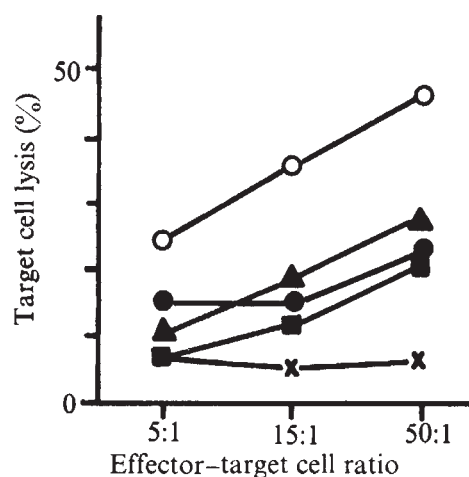


Fig. 1 Cytotoxicity of T cells isolated from the BL biopsy. Cytotoxicity tests were performed in standard V-shaped microplates in a total volume of 140 μ l RPMI medium supplemented with 20% heat-inactivated foetal calf serum. 6×10^3 ⁵¹Cr-labelled cells were used as targets. At the end of a 9-h incubation 100 μ l of supernatant were collected. Cytotoxicity was expressed as the percentage according to the following formula: $(T-SR/M-SR) \times 100$ where T is experimental release, SR is spontaneous release of target cells in the absence of lymphocytes and M is maximal release of target cells lysed with distilled water. Characteristics and origin of target cells are given in Table 1. $\circ$, Oduor (unfractionated 45%, fractionated 5%); Δ , PS-B-1 (unfractionated not done, fractionated 5%); $\blacksquare$, BL biopsy (unfractionated 15%, fractionated 6%); $\times$, K-562 (unfractionated 100%, fractionated 1%); $\bullet$, Kaplan (unfractionated 21%, fractionated 3%). Cytotoxicity of unfractionated and fractionated (see text) peripheral lymphocytes from a normal control, at the highest aggressor: target cell ratio, is given in brackets.

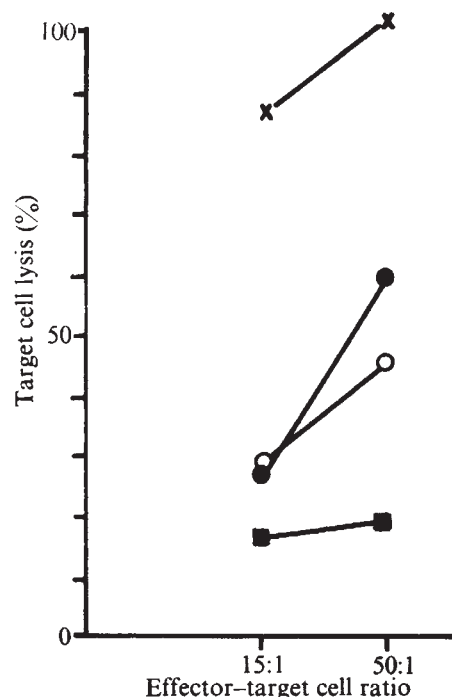
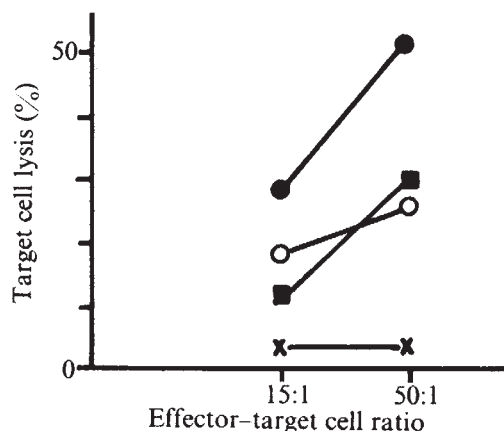


Fig. 2 Cytotoxicity of unfractionated IM lymphocytes. Symbols are the same as in Fig. 1.

T cells isolated from the biopsy killed, in a dose-dependent manner, all three EBV carrying cell lines as well as the autologous tumour biopsy cells (Fig. 1). This cytotoxicity is more impressive than it appears in Fig. 1 as the T cell suspension included 50% unlabelled biopsy tumour cells which probably reduced the indicated ratio of effector target cells by competition with the labelled target cells. A non-EBV-carrying control cell line, K-562, selected for its extreme sensitivity to killing by complement-receptor-bearing lymphocytes, present in all normal people, was hardly killed at all. The capacity of complement-receptor-bearing lymphocytes to unspecifically kill cell lines¹⁰ is illustrated by the high reactivity of the control lymphocytes. Figures 2 and 3 further emphasise this as the nonspecific cytotoxicity against K-562 was removed with the complement-receptor-bearing lymphocytes in contrast to the specific cytotoxicity of the IM cells against the EBV-positive target cells. Furthermore, the T cells from the BL biopsy reacted most strongly against the BL-derived cell line whereas the fractionated IM lymphocytes killed the IM line preferentially. Because of a lack of IM effector cells the PS-B-1 cell line could not be included as a target cell for these cells.

Fig. 3 Cytotoxicity of fractionated IM lymphocytes. Symbols are the same as in Fig. 1.



We draw several conclusions from these experiments. First, BL patients can mount a cell-mediated, T-cell dependent, immune response against their tumours. Second, activated T cells can be recovered from the tumour site and can, in spite of their presence in a large tumour mass, still be cytotoxic against the tumour cells *in vitro*. Third, patients with IM develop killer T cells which may recognise BL tumour cells. This would be of particular interest if cell-mediated immunity could be transferred between humans. If our results hold true, BL (and NPC) patients may benefit from transfer of immunity from patients with IM.

We believe that these results, although preliminary, represent the first demonstration of specific, tumour-reactive T cells isolated from a tumour site in man. The clinical implications, for EBV-associated human diseases, remain to be elucidated.

MIKAEL JONDAL
ERIK SVEDMYR
EVA KLEIN

Department of Tumour Biology,
Karolinska Institutet,
S 104 01 Stockholm 60

SURJIT SINGH

Kenya National Hospital,
Department of Head and Neck Surgery,
Nairobi, Kenya

Received February 24; revised April 11, 1975.

- ¹ Bloom, B. R., Landy, M., and Lawrence, H. S., *Cell. Immun.*, **6**, 331 (1973).
- ² Black, M. M., *Prog. clin. Cancer*, **26** (1965).
- ³ Svedmyr, E., and Jondal, M., *Proc. natn. Acad. Sci. U.S.A.*, (in the press).
- ⁴ Henle, G., Henle, W., and Diehl, V., *Proc. natn. Acad. Sci. U.S.A.*, **59**, 94 (1968).
- ⁵ Klein, G., *The Herpes Viruses* (edit. by Kaplan, A.) (Academic, New York, 1973).
- ⁶ Wolf, H., zur Hausen, H., and Becker, V., *Nature*, **244**, 245 (1973).
- ⁷ Boyum, A., *Scand. J. clin. Lab. Invest.*, **21**, Suppl. 97, 51 (1968).
- ⁸ Jondal, M., Holm, G., and Wigzell, H., *J. exp. Med.*, **136**, 207 (1972).
- ⁹ Jondal, M., *Scand. J. Immun.*, **3**, 739 (1975).
- ¹⁰ Jondal, M., and Pross, H., *Int. J. Cancer*, **15**, 596 (1975).
- ¹¹ Lozzio, C. B., and Lozzio, B. B., *J. natn. Cancer Inst.*, **50**, 535 (1973).

Platelet 5-HT uptake and release stopped rapidly by formaldehyde

UPTAKE of 5-hydroxytryptamine (5-HT) and the thrombin-induced release reaction in platelets are relatively rapid processes at 37 °C, with a linear component of approximately 1 min for uptake¹ and an estimated half life of 6 s for the release reaction^{2,3}. An agent which could essentially stop these processes instantaneously and prevent any further 5-HT exchange across the plasma membrane would therefore be a valuable tool for precise studies of the kinetics of uptake and release. We describe here the treatment of platelets with formaldehyde, a technique which seems to provide such a tool.

In preliminary studies, we investigated the effect of several commonly used electron microscope fixatives on the recovery of labelled 5-HT stored in storage vesicles of human platelets. Vesicular 5-HT stores were specifically labelled with ¹⁴C or ³H labelled 5-HT by incubating platelet-rich plasma for 30 min at 37 °C (refs 4 and 5). Platelets were then spun down, washed and resuspended in an appropriate buffer solution⁵. After the various fixatives were added, the suspension was sampled for the enumeration of platelet-dense bodies⁶ and the platelets then spun down for liquid scintillation counting of labelled material remaining in the platelet pellet⁵.

Osmium tetroxide (OsO₄), potassium permanganate and glutaraldehyde all presented problems in the recovery of vesicle-bound 5-HT (Table 1). Recovery of label from platelets treated with OsO₄ (final concentrations of 0.01% or 0.1%) or potassium permanganate (0.1%) was very poor. The fact that very few dense bodies were seen in whole mounts of platelets fixed in this fashion suggests that both agents may act as releasing agents. Even at 4 °C, where

Table 1 Recovery of labelled 5-HT after treatment with various fixative agents

	Control	Fixative agent			
		Formaldehyde	Glutaraldehyde	OsO ₄	Potassium permanganate
% Recovery of radioactivity, mean ± s.e.m.	100.0	102.0 ± 2.1 (n=22)	77.8 ± 3.4 (n=11)	11.4 ± 4.0 (n=34)	4.2 ± 0.3 (n=20)
No. of dense bodies per 100 platelets	625	642	612	4	6

neither thrombin nor the ionophore A23187 induce release of platelet 5-HT, treatment with 0.1% OsO₄ reduced the amount of labelled 5-HT to 9% of that recovered from control pellets. This phenomenon may account for the relative infrequency of dense bodies seen in osmium-fixed platelets⁷ and perhaps for the variable but generally poor visualisation of granular vesicles in adrenergic nerve terminals fixed with osmium⁸. Although glutaraldehyde (final concentration 0.25%) interfered with extraction of labelled 5-HT from pellets, it did not seem to cause loss of platelet-dense bodies.

Another fixative, formaldehyde, prepared from para-formaldehyde⁹ and used at final concentrations ranging from 0.3% to 1.5%, enabled complete recovery of vesicular labelled 5-HT (Table 1). In addition, formaldehyde did not alter the trapping or binding of either ¹⁴C labelled 5-HT or ³H-inulin by platelets fixed before or after the addition of radioactive material and spun into pellets. About 0.6% of the inulin and a similar percentage of 5-HT (each added at concentrations ranging from 5 × 10⁻⁸ to 2 × 10⁻⁵ M) were recovered from the pellets, with or without the use of formaldehyde. Boullin and O'Brien found a similar percentage of inulin trapped in unfixed platelet pellets¹⁰.

Formaldehyde seemed also not to affect the calcium or phosphorus content of platelet dense bodies, an important consideration since calcium and ATP may be stored¹¹ and are released along with 5-HT^{3,12}. Electron microprobe examination¹³ of dense bodies in control (unfixed) and fixed (0.3% formaldehyde) platelets revealed that the intensities of emitted X rays from both calcium and phosphorus were similar for both treated and control platelets.

Since formaldehyde permits complete recovery of vesicle-bound 5-HT, it can be used to study 5-HT remaining in platelets after various stages of thrombin-induced release. Platelets labelled with ³H- or ¹⁴C labelled 5-HT were resuspended and treated with thrombin³, with formaldehyde added at appropriate times after the addition of thrombin (Fig. 1). Although we have no method to determine exactly how quickly formaldehyde stops the release reaction, the lack of release when formaldehyde was added from 1 to 5 s after thrombin suggests that it may act almost instantaneously. We also observed that formaldehyde stops release triggered by the addition of the ionophore A23187 (ref. 14). As this ionophore does not induce release by interacting with the thrombin receptor¹⁵, it seems that the fixative is not acting simply to inactivate thrombin or the thrombin-receptor interaction. The lag before release is similar to that seen for platelet ATP as demonstrated by other methods¹².

Addition of formaldehyde also seems to be a useful method for rapidly stopping the uptake of labelled 5-HT. Labelled 5-HT was added to platelet suspensions at 37 °C, which were then treated with formaldehyde at appropriate times. A final concentration of 1.5% formaldehyde was required to inhibit uptake immediately and completely. Although the conventional use of an ice bath to chill platelet aliquots to 4 °C may require up to 4 min to inhibit uptake¹ completely, one can obtain reproducible and accurate figures using formaldehyde inhibition of platelet 5-HT uptake during intervals as short as 5 s. Because of the

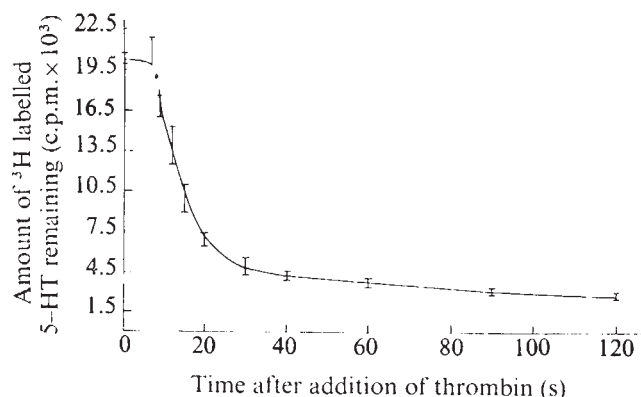


Fig. 1 A least-squares fit curve (—) for the time course of the thrombin-induced release of 5-HT from human platelets. Brackets represent the range of the actual data. Labelled platelets in suspensions at 25 °C were treated with bovine thrombin (final concentration 1 U ml⁻¹). Release was stopped by addition of formaldehyde at various time intervals after addition of thrombin. Although the curve was obtained from the data points by fitting the actual release data with an equation representing the sum of four exponentials, it is not clear that the release reaction may actually be characterised by a four-compartment model.

extremely high affinity of the platelet transport system for 5-HT, this procedure may aid in the accomplishment of more accurate kinetic studies.

Stopping either release or uptake of 5-HT with formaldehyde fixative seems to be a useful tool for studying the kinetics of these processes in detail in platelets and possibly in other suspension systems such as synaptosomes and adrenomedullary chromaffin vesicles. If proved feasible after appropriate recovery experiments, similar techniques may prove valuable with the putative neurotransmitters dopamine and noradrenaline, and possibly with acetylcholine. Further studies of electron microscope fixatives on physiological processes may also reveal interesting anomalies, such as the osmium-induced release of dense bodies observed here.

We thank Dennis Konkel and Alan Weder for technical assistance, and Karen Pettigrew and Clifford Patlak of the Theoretical Statistics and Mathematics Branch, NIMH, for help with the data on 5-HT release.

JONATHAN L. COSTA
DENNIS L. MURPHY

Section on Clinical Neuropharmacology,
Laboratory of Clinical Science,
National Institute of Mental Health,
Bethesda, Maryland 20014

Received March 10; revised April 8, 1975.

- ¹ Tuomisto, J., *J. Pharm. Pharmacol.*, **26**, 92 (1974).
- ² Droller, M. J., *J. Lab. Invest.*, **31**, 197 (1974).
- ³ Detwiler, T. C., and Feinman, R. D., *Biochemistry*, **12**, 282 (1973).
- ⁴ Holmsen, H., Ostvold, A.-C., and Day, H. J., *Biochem. Pharmacol.*, **22**, 2599 (1973).
- ⁵ Murphy, D. L., Colburn, R. W., Davis, J. M., and Bunney, W. E., Jr., *Life Sci.*, **8**, 1187 (1969).
- ⁶ Costa, J. L., Reese, T. S., and Murphy, D. L., *Science*, **183**, 537 (1974).
- ⁷ Sato, T., Herman, L., Chandler, J., Stracher, A., and Detwiler, T. C., *J. Histochem. Cytochem.*, **23**, 103 (1975).
- ⁸ Machado, A. B. M., *Stain Technol.*, **42**, 293 (1967).
- ⁹ Karnovsky, A. B., *J. Cell Biol.*, **27**, 137a (1965).
- ¹⁰ Boullin, D. J., and O'Brien, R. A., *J. Pharm. Pharmacol.*, **20**, 583 (1968).
- ¹¹ Holmsen, H., Day, H. J., Stormshen, H., *Scand. J. Hematol.*, Suppl. **8**, 3 (1969).
- ¹² Detwiler, T. C., and Feinman, R. D., *Biochemistry*, **12**, 2462 (1973).
- ¹³ Costa, J. L., Murphy, D. L., and Tanaka, Y., *Fedn Proc.*, **33**, 269 (1974).
- ¹⁴ Feinman, R. D., and Detwiler, T. C., *Nature*, **249**, 172 (1974).
- ¹⁵ Friedman, F., and Detwiler, T. C., *Biochemistry*, **14**, 1315 (1975).

Rhythmic and phytochrome-regulated changes in transmembrane potential in *Samanea* pulvini

NYCTINASTIC plants such as *Albizia julibrissin*, *Mimosa pudica* and *Samanea saman* have compound leaves with paired leaflets which are usually horizontal (open) during

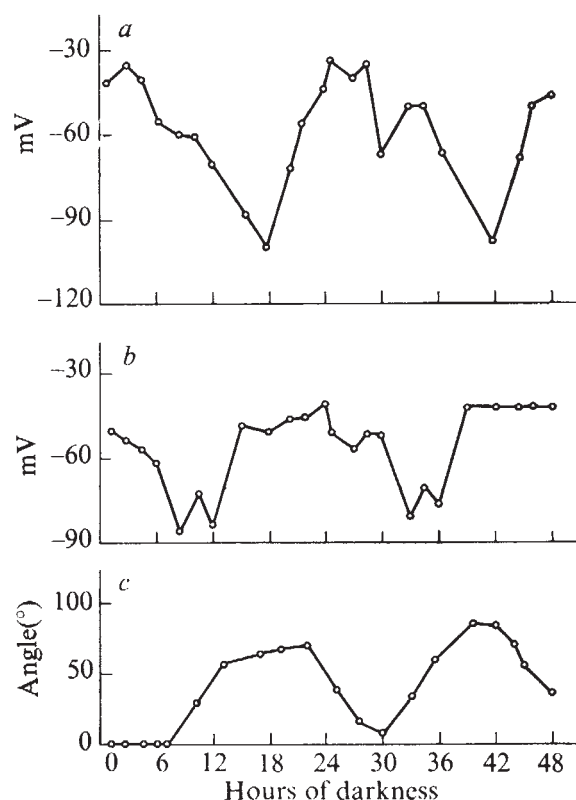
daylight and vertical (closed) at night, oscillating between these positions with a circadian rhythm during long dark periods¹⁻³. The angle of a leaflet is determined by the relative turgour of motor cells on opposite sides of the pulvinus, an organ which subtends the leaflet. Extensor cells are turgid and flexor cells are flaccid when leaflets are open and vice versa when they are closed. This is regulated by movements of potassium^{2,4-8}, which is high in turgid cells and low in flaccid cells. Inhibitors of oxidative phosphorylation and low temperatures impede opening and promote closure, suggesting that active transport predominates during opening, while diffusion is favoured during closure^{4,8-10}.

Light affects K⁺ movements and turgour changes through two photosystems. Prolonged, high intensity white light promotes K⁺ secretion from flexor cells and leaflet opening^{1,7,8}. The onset of white light acts as *zeitgeber* for rhythmic phasing when plants grown in light-dark cycles are transferred to continuous darkness¹⁻³. A blue-absorbing pigment is probably the photoreceptor for these high irradiance responses^{11,12}. Furthermore, brief pulses of red and far red light absorbed by phytochrome regulate nyctinastic (dark-induced) closure and also interact with the biological clock to regulate rhythmic K⁺ flux and leaflet movement^{2,7,8,13-16}. We now have evidence of rhythmic and light-regulated changes in transmembrane potential in motor cells of *Samanea*.

Samanea plants were grown, as before, from seed in constant humidity, constant temperature growth chambers, in cycles of 16 h cool white fluorescent light and 8 h darkness⁴. The usual dark period was extended to 48 h to study rhythmic electrical changes. A green safelight provided illumination for manipulations and measurements during the dark period. Measurements were made at 25 ± 2 °C.

Secondary pulvini from the third to seventh uppermost leaves were cut and mounted in a Lucite chamber and perfused with a solution containing 1 mM KCl, 1 mM Ca (NO₃)₂, 0.25 mM MgSO₄ and a Na phosphate buffer (1 mM, pH 5.6). Cells were impaled immediately after pulvini were

Fig. 1 Rhythmic changes in leaflet angle (c) and transmembrane potential of extensor (b) and flexor cells (a). The average s.d. was ± 8%.



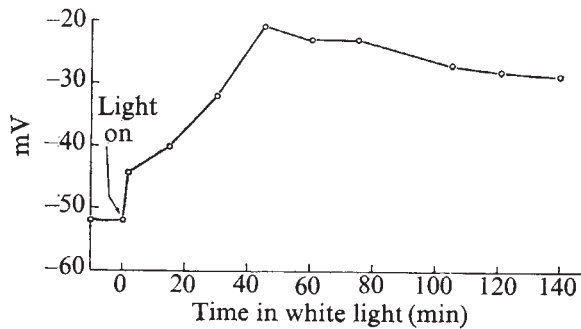


Fig. 2 Transmembrane potential of flexor motor cells of leaflets in cool white fluorescent light (about 10,800 lx). The average s.d. was $\pm 6\%$.

excised, and potentials for Figs 1 and 2 were measured during the first 15–120 s after impalement. In suitable conditions, we ascertained visually the position of the microelectrode within the cell. Because of the high chlorophyll content in these cells, however, this was not always possible. In cases where the electrode was not visible, we ensured that the potential reading was indeed occurring across the membrane by taking transmembrane resistance measurements through the recording electrode. (The necessary circuitry for this procedure is integral to the WPI, M-4A electrometer and details of the theory of operation are outlined in Applications Note No. 1, April 1973. W-P Instruments, Inc. 2600 State Street, Hamden, Connecticut 06517.) Each point on our graphs is an average of four to seven cellular measurements made in three to four pulvini. Potentials that remained stable for less than 15 s were not included. Glass microelectrodes (tip diameter less than $0.5 \mu\text{m}$ and resistance $15\text{--}45 \text{ M}\Omega$) were pulled and filled with 3 M KCl by conventional methods²¹. Each microelectrode was connected by Ag/AgCl wire to a WPI model M-4A electrometer and reference electrodes were earthed. Measurements were made on a chart recorder.

The potential of the motor cells are less negative than those reported for other plant tissues with 1 mM K^+ in the medium^{22,23}. Studies with oats, however, have indicated that low negative potentials are observed in freshly cut tissue and a 24-h incubation is required to reach a more negative potential plateau²⁴.

Figure 1 shows changes of potential in extensor and flexor cells during 48 h of continuous darkness. Leaf angles, measured on intact plants under similar conditions of darkness, were similar to those reported previously. The relative continuity of potential measurements (data taken every 2–3 h for 48 h) provides a kinetic picture of rhythmic oscillations at the membrane. Potentials in both flexor and extensor cells oscillated with circadian periods out of phase by approximately 8 h. The rhythm in potential of the flexor cells was approximately in phase with the leaflet movement rhythm. It is interesting that the flexor cells hyperpolarised during the period described by Satter *et al.* as the energetic portion of the rhythmic cycle, and depolarised during the leaky part of the cycle⁵.

Transmembrane potential in the flexor cells was also monitored during the first few hours of the experimental white light period (Fig. 2). White-irradiated leaflets open more rapidly and completely than those in the dark, presumably as a result of light-promoted K^+ extrusion from flexor cells⁴. White light caused a rapid depolarisation detectable within 1 min. During the first 45 min of the light period, the potential of the flexor cells decreased from -50 to -20 mV , and then repolarised to -30 mV .

Leaflets transferred from white light to darkness closed in about 60–90 min if preirradiated with red light (phytochrome in the P_r form), but more slowly and less completely when preirradiated with far red light (phytochrome in the

P_f form)^{13,15}. We monitored changes in the potential of the flexor cells after the white lights were turned off and the pulvini were exposed to red or far red light. Cells were impaled before the red and far red light treatments to reveal rapid changes. Light sources for phytochrome photoconversions (red light, about 660 nm , at $350 \text{ erg cm}^{-2} \text{ s}^{-1}$ and far red light, about 730 nm , at $900 \text{ erg cm}^{-2} \text{ s}^{-1}$) are described in ref. 25.

The potential of the flexor cells of leaflets treated with red light increased 30 mV (hyperpolarised) in 10 min with no perceptible lag (Fig. 3). Subsequent far red light reversed the red light effect within 90 s, and another exposure to red light restored the hyperpolarisation with 90 s. The potential remained high (more negative) and stable if the plants were darkened after 3 min in red light, and remained low and stable if the plants were darkened after 3 min in far red light (data not included). Thus our electrical data support the contention that phytochrome phototransformations regulate rapid, membrane-associated phenomena²⁶. The lag period was shorter although the time for maximum response was longer after the first red light signal than after subsequent such signals. The first red light treatment, however, followed high irradiance white light, while the second followed far red light. Thus two photosystems (phytochrome and the high irradiance photoreceptor) interacted to regulate the first response, while only phytochrome was involved in the second. The lag after the second red light treatment was probably caused in part by the time required for conversion of appreciable P_r to P_{fr} .

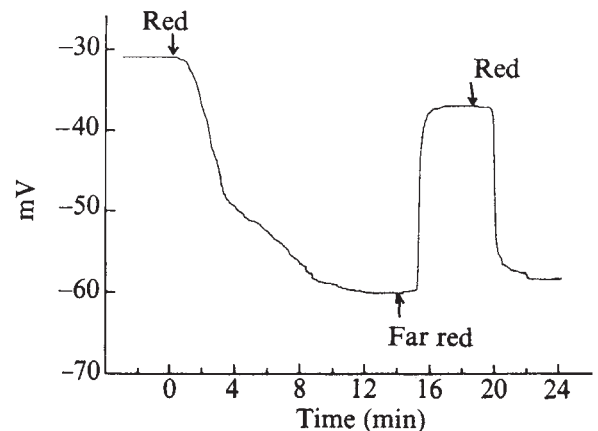


Fig. 3 Phytochrome-controlled changes in the transmembrane potential of flexor motor cells.

Kinetic studies of K^+ movement during nyctinastic closure reveal a large K^+ flux into the flexor motor cells if leaflets are preirradiated with red light, but not if they are preirradiated with far red light (our unpublished data). The K^+ flux is, however, much slower than the change in transmembrane potential, suggesting that other electrical events precede K^+ flux.

Both rhythmic and phytochrome-mediated changes in electrical parameters have been demonstrated in other systems. Spontaneous bursts of nerve action potential in the hypothalamus of the rat¹⁷ and in the eye and parieto-visceral ganglion of *Aplysia*¹⁸ oscillate with circadian periods. Recordings from surface electrodes reveal phytochrome-regulated changes in potential of mung bean root tips and oat coleoptiles^{19,20}. Ours is the first demonstration that the biological clock and two different pigment systems interact to regulate the membrane potential of the same cells. Evidence of rhythmic oscillations in electrical parameters of the membrane support the suggestion that rhythms in the composition or properties of membranes provide a basis for endogenous oscillations in various organisms^{4,9,27–31}. Experiments with light stimuli provide further evidence for this view. Both white light, which entrains the leaflet movement

in *Samanea*¹, and the membrane-localised chromoprotein phytochrome³²⁻³⁴ which has been shown to initiate rhythms in other plants^{35,36}, regulate membrane electrical properties in *Samanea* pulvini.

This work was supported by grants from the US National Science Foundation to A. W. Galston and B. Etherton, both of whom we thank for reading the manuscript and making helpful suggestions.

RICHARD RACUSEN

Botany Department,
University of Vermont,
Burlington, Vermont 05401

RUTH L. SATTER

Biology Department,
Yale University,
New Haven, Connecticut 06520

Received February 27, 1975.

- ¹ Palmer, J. H., and Asprey, G. F., *Planta*, **51**, 757-769 (1958).
- ² Satter, R. L., and Galston, A. W., *Science*, **174**, 518-520 (1971).
- ³ Koukkari, W. L., Halberg, F., and Gordon, S. A., *Pl. Physiol.*, **Lancaster**, **51**, 1084-1088 (1973).
- ⁴ Satter, R. L., Geballe, G. T., Applewhite, P. B., and Galston, A. W., *J. gen. Physiol.*, **64**, 413-430 (1974).
- ⁵ Toriyama, H., *Cytologia*, **20**, 367-377 (1955).
- ⁶ Allen, R. D., *Pl. Physiol.*, **Lancaster**, **44**, 1101-1107 (1969).
- ⁷ Satter, R. L., Marinoff, P., and Galston, A. W., *Am. J. Bot.*, **57**, 916-926 (1970).
- ⁸ Satter, R. L., and Galston, A. W., *Pl. Physiol.*, **Lancaster**, **48**, 740-746 (1971); *BioScience*, **23**, 407-416 (1973).
- ⁹ Satter, R. L., Applewhite, P. B., and Galston, A. W., *Pl. Physiol.*, **Lancaster**, **54**, 280-285 (1974).
- ¹⁰ Satter, R. L., Applewhite, P. B., Kreis, D. J., and Galston, A. W., *Pl. Physiol.*, **Lancaster**, **52**, 202-207 (1973).
- ¹¹ Fondeville, J. C., Schneider, M. J., Borthwick, H. A., and Hendricks, S. B., *Planta*, **75**, 228-238 (1967).
- ¹² Evans, L. T., and Allaway, W. G., *Aust. J. biol. Sci.*, **25**, 885-893 (1972).
- ¹³ Satter, R. L., Geballe, G. T., and Galston, A. W., *J. gen. Physiol.*, **64**, 431-442 (1974).
- ¹⁴ Jaffe, M. J., and Galston, A. W., *Planta*, **77**, 135-141 (1967).
- ¹⁵ Sweet, H. G., and Hillman, W. S., *Physiol. Pl.*, **22**, 776-786 (1969).
- ¹⁶ Fondeville, J. C., Borthwick, H. A., and Hendricks, S. B., *Planta*, **69**, 357-364 (1966).
- ¹⁷ Schmitt, M., *Am. J. Physiol.*, **225**, 1096-1101 (1973).
- ¹⁸ Jacklet, J. W., *Science*, **164**, 562-563 (1969).
- ¹⁹ Jaffe, M. J., *Science*, **162**, 1016-1017 (1968).
- ²⁰ Newman, I. A., and Briggs, W. R., *Pl. Physiol.*, **Lancaster**, **50**, 687-693 (1972).
- ²¹ Tasaki, K., Tsukahara, Y., Ito, S., Wayne, M. J., and Yu, W. Y., *Physiol. Behav.*, **3**, 1009-1010 (1968).
- ²² Etherton, B., *Pl. Physiol.*, **Lancaster**, **38**, 581-585 (1963).
- ²³ Higinbotham, N., Graves, J. S., and Davis, R. F., *J. Membrane Biol.*, **3**, 210-222 (1970).
- ²⁴ Macklon, A. E. S., and Higinbotham, N., *Pl. Physiol.*, **Lancaster**, **43**, 888-892 (1968).
- ²⁵ Racusen, R. H., and Miller, K., *Pl. Physiol.*, **Lancaster**, **49**, 654-655 (1972).
- ²⁶ Hendricks, S. B., and Borthwick, H. A., *Proc. natn. Acad. Sci. U.S.A.*, **58**, 2125-2131 (1967).
- ²⁷ Njus, D., Sulzman, F. M., and Hastings, J. W., *Nature*, **248**, 116-119 (1974).
- ²⁸ Strumwasser, F., in *Circadian clocks* (edit. by Aschoff, J.), 442-462 (North-Holland, Amsterdam, 1969).
- ²⁹ Lickey, M. E., *J. comp. Physiol. Psych.*, **68**, 9-17 (1969).
- ³⁰ Bunning, E., and Moser, I., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2732-2733 (1972).
- ³¹ Sweeney, B. M., *Pl. Physiol.*, **Lancaster**, **53**, 337-342 (1974).
- ³² Haupt, W., Mortel, G., and Winkelkemper, I., *Planta*, **88**, 183-186 (1969).
- ³³ Boisard, J., Marmé, D., and Briggs, W. R., *Pl. Physiol.*, **Lancaster**, **54**, 272-276 (1974).
- ³⁴ Quail, P. H., Marmé, D., and Schafer, E., *Nature new Biol.*, **245**, 189-190 (1973).
- ³⁵ Lörcher, L., *Z. Bot.*, **46**, 209-241 (1958).
- ³⁶ Hillman, W. S., *Photochem. Photobiol.*, **21**, 39-47 (1975).

Inhibition of Fc receptors on a murine lymphoid cell line by cholera exotoxin

AFTER binding to its receptor, Gm1 ganglioside^{1,2}, cholera exotoxin has been demonstrated to be a potent activator of the adeny cyclase system in several cell systems. The response to exotoxin is related to an increase in intracellular cyclic 3', 5' adenosine monophosphate (cyclic AMP). Agents that increase intracellular cyclic AMP have been shown to suppress certain immunological responses, including histamine release³, lysosome degranulation⁴, mitogenic stimulation⁵, T lymphocyte rosette formation⁶ and antibody-dependent cellular cytotoxicity⁷. We now report that cholera exotoxin can inhibit the expression of plasma membrane receptors for the Fc portion of IgG in a murine lymphoid cell system.

We used two cell lines derived from a spontaneous myeloma in the BALB/c mouse (PU-5). These two lines were cloned into one line bearing (Fc+) and one line lacking (Fc-) a plasma membrane receptor for the Fc portion of IgG (S. D. D. and J. Buxbaum, unpublished observation). The lines were maintained in our laboratory for 1 yr as suspension cultures at

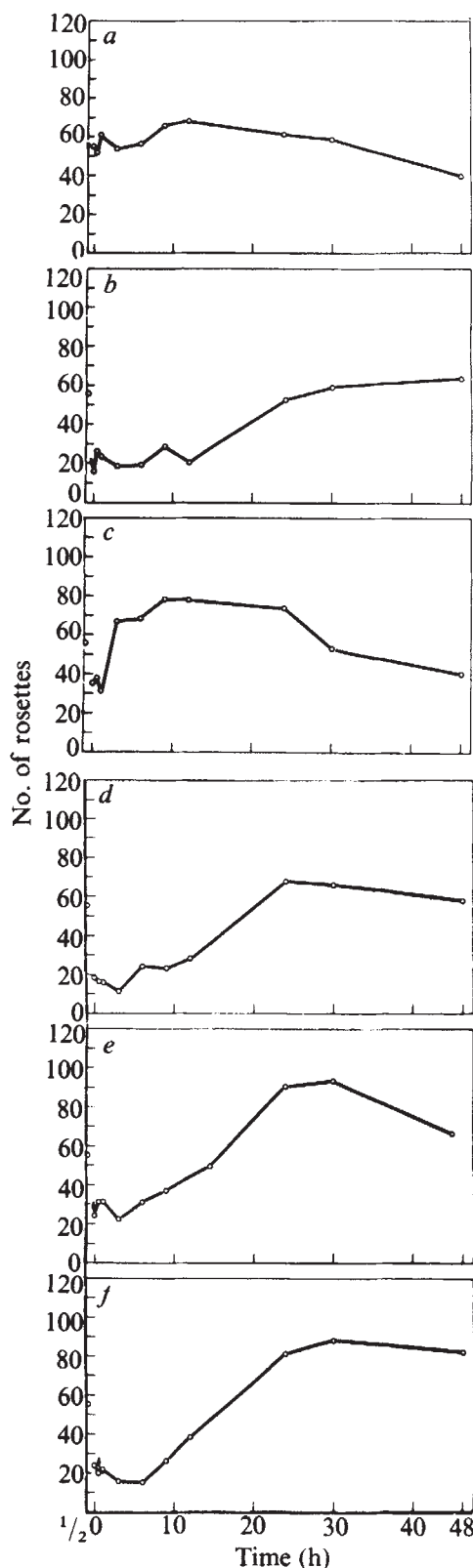


Fig. 1 Cholera exotoxin ($10 \mu\text{g ml}^{-1}$), cholera toxoid ($10 \mu\text{g ml}^{-1}$), aminophylline (10^{-3} M) and dibutyryl cyclic GMP (10^{-6} M) were incubated with Fc+ and Fc- cells at 2×10^6 cells per ml in a 37°C incubator in a $5\% \text{ CO}_2$ atmosphere. (Arrow indicates time point of exotoxin addition.) At designated times 1×10^6 cells per culture were removed and an FA rosette assay was performed. Viability determined was by Trypan blue dye exclusion was always greater than 70% . a, Control; b, cholera toxin + aminophylline; c, cholera toxoid; d, cholera toxin; e, cholera toxin + cyclic GMP; f, toxoid + 30-min pulse with toxin.

Table 1 Cholera toxin dose response of Fc+ PU-5 cell line

Cholera toxin ($\mu\text{g ml}^{-1}$)	3 h		24 h	
	Cyclic AMP (pmol per 10^6 cells)	No. rosettes*	Cyclic AMP (pmol per 10^6 cells)	No. rosettes*
—	0.16	65	0.54	78
0.01	1.4	71	0.88	83
0.1	1.9	70	1.4	74
1.0	2.6	66	1.4	85
10.0	2.0	18	1.8	77

*Per 200 cells.

S.d. ± 4.8 rosettes; the difference in rosettes between toxin and control at 3 h was statistically significant ($P < 0.001$). A s.d. of ± 0.28 pmol per 10^6 cells was observed in cyclic AMP assays.

37 °C in a 5% CO₂ atmosphere at a concentration of approximately 1.5×10^6 cells per ml in Dulbecco's modified essential medium containing 20% horse serum. Fc receptor activity was assayed using 0.5% sheep erythrocyte suspension coated with a 1:400 dilution of rabbit Forssman antibody (EA) and cells binding at least three erythrocytes were considered positive; at least 200 cells were counted⁸. Cholera exotoxin (Lot 0172) prepared by R. A. Finkelstein⁹ under contract for the National Institute of Allergy and Infectious Diseases, and cholera toxoid (Lot 11101) prepared by treatment with glutaraldehyde¹⁰ were obtained from C. Miller. Aminophylline and dibutyryl cyclic GMP were purchased from Sigma Chemical Co.. Cyclic AMP¹¹ was analysed using a radioimmunoassay kit (Schwarz-Mann) and intracellular cyclic AMP was extracted in hot sodium acetate⁵. For morphological studies, cells were fixed in glutaraldehyde, followed by 25% glycerol¹², fractured at -100 °C in a Balzer's 301 freeze etch machine, shadowed with platinum-carbon and the replicas were examined in a Siemens 102 electron microscope.

Cholera exotoxin ($10 \mu\text{g ml}^{-1}$) had a significant inhibitory effect on rosette formation (Fig. 1), which was optimal after 3 h of incubation. Fc receptor activity was reduced for 12 h, gradually recovering during the next 6–10 h. Aminophylline (10^{-3} M) potentiated the effect of toxin on Fc receptor activity and dibutyryl cyclic GMP (10^{-6} M) slightly inhibited this effect. Dibutyryl cyclic GMP (10^{-6} M) enhanced rosette activity in the absence of exotoxin. Inhibition of rosette activity by toxoid differed from that by toxin in time course and degree. Optimal inhibition with toxoid occurred after 1 h, and after 3 h inhibition could no longer be detected. The kinetics of inhibition of the Fc+ line by toxoid followed by toxin 30 min later resembled the response to toxin alone. This effect may be related to competition for the Gml receptor, the toxin having a greater binding affinity than toxoid. Fc- cells gave 0–3% rosettes in the presence or absence of toxin and were sensitive at 10 ng ml^{-1} to activation of their adenyl cyclase system. With Fc+ cells, although as little as 10 ng of exotoxin causes increased intracellular cyclic AMP, a concentration of $10 \mu\text{g ml}^{-1}$ was required to inhibit rosette activity (Table 1). After 24 h, intracellular cyclic AMP levels were still elevated, although the Fc receptor

activity was not inhibited. Supernatants of 24-h cultures of exotoxin-treated Fc+ cells inhibited rosette formation (Table 2). Toxin-treated cells which demonstrated recovery of Fc activity were refractory to repeated exotoxin-induced inhibition of rosette activity (Table 2). Freeze fracture studies indicated an increase in intramembranous particles after incubation of either cell line with cholera exotoxin (Fig. 2a and b). Quantitative studies of this phenomenon are in progress. Our findings demonstrated that treatment of a lymphoid cell line with cholera exotoxin ($10 \mu\text{g ml}^{-1}$) inhibits Fc receptor activity, with subsequent recovery. The amount of toxin required for this effect is approximately 1,000 times that required to increase intracellular cyclic AMP. After addition of cholera toxin, intracellular cyclic AMP increases in both Fc+ and Fc- cell lines, showing that these cells are sensitive to this action of the toxin.

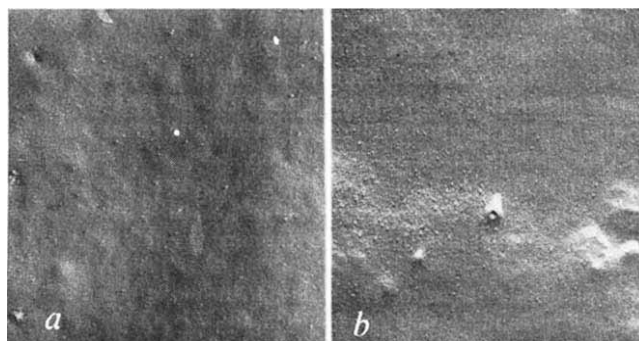


Fig. 2 Freeze fracture replicas of Fc+ PU-5 line. ($\times 52,500$) a, Control (3h); b, cholera exotoxin ($10 \mu\text{g ml}^{-1}$, 3h). Note increased number of intramembranous particles in (b).

Our findings indicate that cholera exotoxin inhibits Fc receptor activity, elevates intracellular cyclic AMP and may cause an increase in intramembranous particles in a murine lymphoid cell line. The potentiation of cholera exotoxin inhibition of Fc receptor activity by aminophylline and the antagonistic effect exerted by dibutyryl cyclic GMP suggests that cyclic nucleotide balance may have a dynamic role in membrane receptor expression. The dose-response relationship observed suggests that cyclic AMP levels may not directly effect Fc receptor expression, but that the inhibition of receptor activity may be associated with a secondary or tertiary effect of the cyclic nucleotides, perhaps mediated through protein kinases.

This work was supported by grants from the US Public Health Service, the National Leukemia Association, the American Lung Association and the Graduate School of the University of Minnesota.

STEVEN H. ZUCKERMAN
STEVEN D. DOUGLAS

Departments of Medicine and Microbiology,
University of Minnesota Medical School,
Minneapolis, Minnesota 55455

Table 2 Fc receptor activity after 24 h incubation with cholera toxin

	No. rosettes
Cells (toxin 24 h)	71
Cells (toxin 24 h) + toxin (second pulse)	70
Control cells + control supernatant (24 h)	87
Control cells + toxin-treated cell supernatant (24 h)	29
Control cells + toxin	26

Fc+ at 2×10^6 cells per ml were incubated for 24 h in presence (toxin treated) or absence (control) of exotoxin ($10 \mu\text{g ml}^{-1}$). Cultures were centrifuged, supernatants (10 ml) control and from toxin-treated cells were decanted and 2×10^7 control cells were resuspended in each of the two supernatants. Toxin-treated cells were resuspended in fresh media and a second pulse with toxin was introduced. All cultures were incubated for an additional 1 h at 37 °C and rosette assay was performed. S. d. ± 10.3 rosettes.

Received February 28; accepted April 21, 1975.

- ¹ Cuatrecasas, P., *Biochemistry*, **12**, 3547-3558; 3558-3566; 3567-3577 (1973).
- ² Bennett, V., O'Keefe, E., and Cuatrecasas, P., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 33-37 (1975).
- ³ May, C. D., Levine, B. B., and Weissmann, G., *Proc. Soc. exp. Biol. Med.*, **133**, 758-763 (1970).
- ⁴ Zurier, R. B., Weissmann, G., Hoffstein, S., Kammerman, S., and Tai, H. H., *J. clin. Invest.*, **53**, 297-309 (1974).
- ⁵ DeRubertis, F. R., Zenser, T. V., Adler, W. H., and Hudson, T., *J. Immun.*, **113**, 151-161 (1974).
- ⁶ Chisari, F. V., and Edgington, T. S., *J. exp. Med.*, **140**, 1122-1126 (1974).
- ⁷ Gale, R. P., and Zigelboim, J., *J. Immun.*, **113**, 1793-1800 (1974).
- ⁸ Douglas, S. D., Schmidt, M. E., and Siltzbach, L. E., *Immun. Commun.*, **1**, 25-38 (1972).
- ⁹ Finkelstein, R. A., and Lospalluto, J. R., *J. infect. Dis.*, **121**, S63-S72 (1970).
- ¹⁰ Rappaport, R. S., Bonde, G., McCann, T., Rubin, B. A., and Tint, H., *Infect. Immunity*, **9**, 304-317 (1974).
- ¹¹ Steiner, A. L., Parker, C. W., and Kipnis, D. M., *J. biol. Chem.*, **247**, 1106-1113 (1972).
- ¹² Pinto da Silva, P., *J. Cell. Biol.*, **53**, 777-787 (1972).

Fc receptors on mouse placenta and yolk sac cells

MACROMOLECULAR exchange across the foeto-maternal interface in the pregnant mammal is in some species confined to the placenta, whereas in others such as the rabbit, rat and mouse, the yolk sac is also involved¹. Whatever the route, it is well established that whereas most proteins are broken down during their passage into the foetus, a small proportion of certain immunoglobulins is transferred to the foetal circulation intact. This has important implications not only for the transmission of passive immunity but also for the access of potentially deleterious maternal antibody directed against foetal antigens.

Immunoglobulin transport into the foetus is highly selective with respect to both species of origin and class of antibody¹, and a considerable amount of discussion has centred on the possible mechanisms involved. Brambell^{1,2} hypothesised that the transmission of intact antibody might depend on its attachment to cell-surface Fc receptors, which on subsequent endocytosis could afford protection from lysosomal enzyme action. Using antibody-coated red blood cells (RBC) in a rosetting technique successfully used by others to detect Fc receptors on macrophages and B lymphocytes^{3,4}, we have now demonstrated the presence of these receptors on both placental and yolk sac cells in the mouse.

Yolk sac membranes were dissected from mice on days 15-18 of gestation and cell suspensions prepared as described previously⁵. Contaminating erythrocytes were lysed by treatment with 0.83% ammonium chloride⁶, and the preparations washed in a large volume of phosphate-buffered saline (PBS) and resuspended in medium RPMI and 10% foetal calf serum

Table 1 Binding of antibody-coated RBC to mouse yolk sac cells

Group	Mouse strain	Antibody	RBC	Mean % rosettes* ± s.d.
1	C57BL	C57 anti-A†	Mouse A strain	22.2 ± 8.0
	C57BL	—	Mouse A strain	1.5 ± 1.0
2	A	Human anti-D‡	Human D	15.2 ± 2.8
	A	—	Human D	2.8 ± 1.3
3	C57BL	Human anti-D	Human D	20.3
	C57BL	—	Human D	1.6
4	A	Rabbit anti-mouse§	Mouse	13.9
	A	—	Mouse	1.0
5	C57BL	Rabbit anti-mouse	Mouse	35.4
	C57BL	—	Mouse	1.7

*Mean percentage rosettes = Mean (No. of rosettes/No. of endo-derm cells) × 100.

†C57 anti-A serum was raised by six weekly intraperitoneal injections of A strain spleen cells into C57BL mice. Bled 6 d after last injection.

‡Human anti-D serum was from a rhesus alloimmunised individual.

§Rabbit anti-mouse serum was prepared by intramuscular injection of 5 × 10⁷ DBA mastocytoma cells in FCA, followed by several intravenous injections of 4 × 10⁷ DBA mastocytoma cells. Bled 10 d after the last injection.

Group 1: mean of five experiments.

Group 2: mean of three experiments.

Table 2 Effect of removal of the Fc portion of immunoglobulin on the formation of rosettes by mouse yolk sac cells

Indicator RBC	Mean % rosettes* ± s.d.
IgG-coated mouse A strain†	20.4 ± 11.4
F(ab') ₂ -coated mouse A strain‡	1.3 ± 0.6

*Mean of three experiments.

†Prepared by DEAE chromatography of rabbit anti-mouse serum and adjusted to a protein concentration of 0.4 mg ml⁻¹.

‡Prepared by pepsin digestion of the immunoglobulin fraction of rabbit anti-mouse serum, separated on Sephadex G-150 and adjusted to a protein concentration of 0.4 mg ml⁻¹. F(ab')₂ coating of the RBC was tested by agglutination with sheep anti-rabbit immunoglobulin.

to a concentration of 1 × 10⁶ ml⁻¹. Aliquots (0.5) ml of the suspensions were dispensed into the wells of a leukocyte migration plate (Sterilin) and incubated overnight in 5% CO₂ in air at 37 °C. Cultures were then washed by inversion in a Petri dish containing PBS, and 0.4 ml of a 2% suspension of indicator RBC added to each well. After 60 min incubation at room temperature, the cultures were washed and the cells binding RBC ('rosettes') enumerated using an inverted microscope. In five separate experiments the percentage of cells binding RBC coated with mouse alloantibody was significantly different from control cultures with non-coated indicator RBC (*P* < 0.01) (Table 1, group 1). This indicates that cultured yolk sac cells possess cell-surface receptors which, like the Fc receptors on macrophages, can bind antibody complexed with its antigen. That these receptors can also bind heterologous antibody in a similar manner was shown by the use of RBC coated with either human or rabbit antibody (Table 1, groups 2-5). The binding seems to be specific for immunoglobulin since rosetting did not occur with indicator RBC coupled to bovine serum albumin.

To confirm that rosette formation was dependent on Fc receptors, the test was carried out using immunoglobulin from which the Fc component had been removed. The antigen-binding F(ab')₂ fragment was prepared by pepsin digestion of the immunoglobulin fraction of rabbit anti-mouse serum⁷. Yolk sac cells incubated with indicator RBC coated with this F(ab')₂ preparation were unable to form rosettes, whereas those incubated with RBC coated with intact immunoglobulin were able to do so (Table 2).

The ability of certain lymphocyte populations to form Fc rosettes can be specifically inhibited by pretreatment with alloantisera directed against their histocompatibility antigens⁴, possibly suggesting a close association, on the cell membrane, of histocompatibility antigens and Fc receptors. To determine whether this was also the case with yolk sac cells, monolayer cultures of A strain yolk sac were treated for 60 min at 37 °C with C57BL anti-A strain serum before washing and incubation with indicator cells. Alloantisera pretreatment caused a marked inhibition of the number of cells forming rosettes compared with control cultures pretreated with normal C57BL serum. On the other hand, the rosetting ability of C57BL yolk sac cells was unaffected by pretreatment with the same anti-serum (Table 3).

Table 3 Inhibition of rosette formation by treatment of yolk sac cells with alloantisera

Mouse strain	Experiment 1	% Inhibition* Experiment 2	Experiment 3
A	90.4	80.9	84.9
C57BL	ND	16.7	0.7

*% Inhibition 100 - (% rosettes in C57 anti-A pretreated cultures/% rosettes in C57BL normal serum pretreated cultures) × 100.

Cultures pretreated with serum diluted 1 to 5 in RPMI for 60 min at 37 °C.

Rosette formation carried out using mouse A strain RBC coated with C57 anti-A serum as indicator RBC.

ND, Not determined.

In the mouse little is known of the relative contribution of the yolk sac and placental routes to immunoglobulin transmission from mother to foetus. If the placenta is important, cells bearing Fc receptors should also be detectable in this organ. This possibility was investigated by the use of cultures of whole 15–18-d placentae prepared in a manner similar to that described for the yolk sac. After washing, overnight cultures were incubated with indicator RBC as before. The results indicate that cells bearing Fc receptors are present in placental preparations and that, like those of the yolk sac, they are capable of binding both homologous and heterologous immunoglobulin (Table 4).

Since the placenta is a compound organ to which both mother and foetus contribute, it is not possible to conclude that the rosette-forming cells were foetal in origin. To investigate their identity we used the ability of alloantisera to inhibit rosette formation specifically in experiments in which the foetal and maternal components of the placental preparations were of different genotype. Placentae in which the foetal component was A strain and the maternal component C57BL, were obtained by transferring A strain blastocysts to the uteri of pseudopregnant C57BL females on day 2 *post coitum*⁸ and allowing development to proceed to day 16. Cell cultures were prepared as before and pretreated with alloantisera before rosetting as described for yolk sac. As shown in Table 5, rosette formation by A strain placentae from A strain mothers is almost completely inhibited by pretreatment with alloantisera. Rosette formation by A strain placentae from C57BL mothers also shows considerable inhibition after pretreatment, indicating that many of the rosetting cells in these preparations are A strain and therefore foetal in origin. Pretreatment of C57BL placenta from C57BL mothers had no effect on rosette formation.

The Brambell hypothesis for the transfer of immunoglobulin to the foetus predicts that receptors for these proteins are present either on the cell surface or within pinocytotic vesicles. Furthermore, the preferential transfer of labelled Fc as opposed to F(ab')₂ fragments to the rabbit foetus has led to the suggestion that these receptors have specificity for the Fc portion of the antibody molecule⁹. The present findings indicate that such receptors exist on the surface of certain cells in both the yolk sac and placenta of the mouse.

Table 4 Binding of antibody-coated RBC to mouse placental cells

Group	Mouse strain	Antibody	RBC	Mean rosettes per field* ±s.d.
1†	A	C57 anti-A	Mouse A strain	24.9±3.8
	A	—	Mouse A strain	0.4±0.2
2	C57BL	C57 anti-A	Mouse A strain	55.3
	C57BL	—	Mouse A strain	0.3
3	A	Human anti-D	Human D	12.6
	A	—	Human D	0.0
4	C57BL	Human anti-D	Human D	23.8
	C57BL	—	Human D	0.0

*Number of rosettes per microscope field counted. Minimum of six fields scored.

†Mean of two experiments.

It is of interest to consider the precise nature of these receptor-bearing cells. On the basis of their size and morphological characteristics those in the yolk sac preparations were identified as endodermal; this was supported by two further observations. Isolated conceptuses, with their surrounding yolk sac intact, were exposed briefly to a suspension of neutral red before preparation of the membrane. This procedure labelled the pinocytotically-active outer endodermal cells in contact with the dye, and allowed their identification as rosette-forming cells in subsequent cytocentrifuged preparations. Final confirmation was obtained by the use of cultures of pure endoderm prepared by the technique described previously⁵ which were also able to form rosettes. Many of the rosetting cells from the

Table 5 Inhibition of rosette formation by alloantisera treatment of placental cells derived from normal and transferred mouse embryos

Mouse strain		% Inhibition*			
Foetus	Mother	Experiment 1	Experiment 2	Experiment 3	Experiment 4
A	A	96.2	87.2	86.5	97.2
C57BL	C57BL	ND	17.1	—27.4	—32.3
A†	C57BL	ND	ND	78.3	52.2

*% Inhibition = 100 — (No. rosettes per field in C57 anti-A pretreated cultures/No. rosettes per field in C57BL normal serum pretreated cultures) × 100.

Cultures pretreated with serum diluted 1 to 5 in RPMI for 60 min at 37 °C.

Rosette formation carried out using mouse A strain RBC coated with C57BL anti-A serum as indicator RBC.

ND, Not determined.

placenta were foetal in origin, as shown by the egg transfer experiments, and from their frequency and general morphology it seems reasonable to suggest that they are trophoblastic, although the involvement of cells with similar characteristics, such as macrophages, cannot be discounted. The possibility of their being endodermal elements from the crypts of Duval¹¹ was minimised by routine removal of this region of the placenta during preparation. Note that many cells in both foetal tissues did not form rosettes. This may be related to the regional specialisation which can be observed in the yolk sac, and to the diversity in trophoblastic cell populations in the placenta¹².

It is known that heterologous antisera can produce teratogenic effects on rat embryos *in vivo* and *in vitro* as a secondary consequence of interference with yolk sac nutritive support¹³. The specific effect of alloantisera on cell-surface function, as shown by inhibition of receptor-dependent rosette formation, suggests that maternal antibody may also have the ability to influence foetal membrane transport systems. An explanation for the suggested association between human congenital abnormalities and elevated HL-A antibody levels in pregnancy¹⁴ could be considered in these terms.

Apart from their significance for the transfer of immunoglobulin the presence of Fc receptors on placental cells may contribute to the protection of the conceptus as an intrauterine allograft. Since antigen-antibody complexes have been implicated in the suppression of cell-mediated immune reactions¹⁵, the possession of Fc receptors by trophoblastic elements in the placenta may allow the attachment of such complexes, thereby interfering with antigen recognition by maternal immune cells.

We thank Dr R. F. Searle for performing the egg transfers, Mr R. G. Oldroyd for help with the preparation of immunoglobulin fractions and Miss V. A. Merry for assistance. This work was supported by a grant from The Rockefeller Foundation.

JOANNA ELSON
E. J. JENKINSON
W. D. BILLINGTON

*Reproductive Immunology Group,
Department of Pathology,
The Medical School,
University of Bristol,
Bristol BS8 1TD, UK.*

Received February 21; accepted April 22, 1975.

- Brambell, F. W. R., *Transmission of Passive Immunity from Mother to Young*, (North-Holland, Amsterdam and London, 1970).
- Brambell, F. W. R., *Lancet*, ii, 1087 (1966).
- Berken, A., and Benacerraf, B., *J. exp. Med.*, **123**, 119 (1966).
- Parish, C. R., and Hayward, J. A., *Proc. R. Soc.*, **B187**, 47 (1974).
- Jenkinson, E. J., and Billington, W. D., *J. Reprod. Fert.*, **41**, 403 (1974).
- Hayry, P., and Defendi, V., *Clin. exp. Immun.*, **6**, 345 (1970).
- Nisonoff, A., *Meth. med. Res.*, **10**, 134 (1964).
- Dickmann, Z., in *Methods in Mammalian Embryology*, (edit. by Daniel, J. C.), 133 (Freeman, San Francisco, 1971).
- Brambell, F. W. R., *Proc. R. Soc.*, **B151**, 478 (1960).
- Hemmings, W. A., and Williams, E. W., *Proc. R. Soc.*, **B187**, 209 (1974).
- Wild, A. E., in *Lysosomes in Biology and Pathology* (edit. by J. T. Dingle), 169 (North-Holland, Amsterdam and London, 1973).

- ¹² Billington, W. D., in *Immunobiology of Trophoblast* (edit. by Edwards, R. G., Howe, C. W. S., and Johnson, M. H.), (Cambridge University Press, in the press).
- ¹³ New, D. A. T., and Brent, R. L., *J. Embryol. exp. Morph.*, **27**, 543 (1972).
- ¹⁴ Terasaki, P. I., Mickey, M. R., Yamazaki, J. N., and Vredevoe, D., *Transplantation*, **9**, 538 (1970).
- ¹⁵ Hellström, K. E., and Hellström, I., *Adv. Immun.*, **18**, 209 (1974).

New calcium-mobilising agent

DURING the past three years calcium ionophores have been increasingly used to study calcium-dependent mechanisms, for example, excitation-contraction coupling¹ and stimulus-secretion coupling²⁻⁵. They seem to act by increasing the cytoplasmic ionised calcium concentration, but the mechanism of action does not seem to be identical in the different tissues studied. The calcium ionophore A23187 transports calcium from the extracellular space and intracellular sources towards the cytosol^{2,3} whereas another ionophore X537A (Lasalocid) seems to release calcium from intracellular binding sites only^{1,5}. In addition, X537A does not seem to affect all the tissues studied so far—possibly because it does not readily penetrate cell membranes.

In the present study we have investigated the effects of Br-X537A, a derivative of Lasalocid, on hormone release from and ⁴⁵Ca movements in the neurohypophysis. The results show that this ionophore is much more potent than X537A. In the absence of any other stimulus the addition of Br-X537A to the incubation medium induced a 25-fold increase in hormone release (Fig. 1a). The ionophore can also induce a twelve-fold increase in hormone secretion at a concentration as low as 2 µg ml⁻¹ (Fig. 2). No attempt was made to study the hormone release at lower ionophore concentration. This release cannot have been caused by disruption of the cell membrane

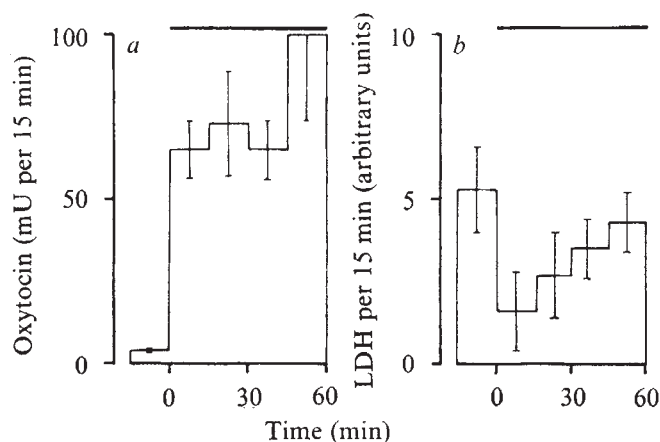


Fig. 1 Effect of Br-X537A on oxytocin release from the rat neurohypophysis (a); and on release of lactate dehydrogenase from glands exposed to the ionophore (b). After isolation of the neurohypophyses, groups of four glands were incubated for 30 min in Locke solution and then transferred to a Na-free solution for successive periods of 15 min. A Na-free solution was used to avoid dimer formation by the ionophore, which tends to occur in solutions containing sodium. Ionophore was added at a concentration of 20 µg ml⁻¹ at time 0. Dimethylsulphoxide (1%) was present throughout the experiment. The oxytocin released into the incubation medium was determined by a bioassay¹¹ which was not affected by either dimethylsulphoxide or ionophore in the concentration used. Each column in (a) represents the oxytocin released during 15 min collection period (mean ± s.e., *n* = 4). b, Amount (arbitrary units) of LDH released into the medium (mean ± s.e., *n* = 4). This cytoplasmic enzyme was assayed by the method of Neilands¹². Br-X537 was present during the periods indicated by the heavy horizontal bars. The composition of the Locke's solution was: NaCl, 150 mM; CaCl₂, 2.2 mM; MgCl₂, 1 mM; KHCO₃, 5.6 mM; glucose, 10 mM. It was equilibrated with 5% CO₂ in O₂. In the Na-free solution choline chloride (150 mM) was added to maintain the tonicity.

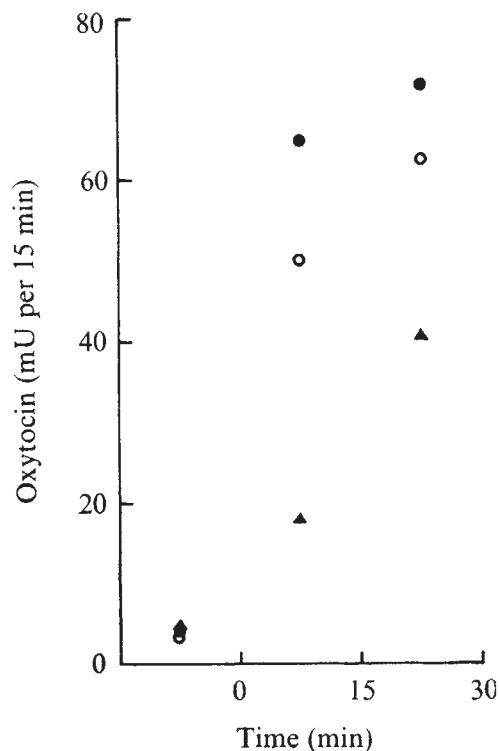


Fig. 2 Effect of ionophore concentration on oxytocin output ●, 20 µg ml⁻¹; ○, 10 µg ml⁻¹; ▲, 2 µg ml⁻¹.

and leakage of cytoplasm into the medium since the concentration of a cytoplasmic enzyme lactate dehydrogenase (LDH) appearing in the medium did not increase (Fig. 1b). It can be concluded that Br-X537A probably induces hormone release by exocytosis. Neurohypophysial hormone release, whether evoked by electrical stimulation⁶ or a high potassium concentration⁷ is associated with an uptake of calcium from the extracellular space. If the drug D600 (10⁻⁴ M), which is known to block calcium entry and hormone release from the neurohypophysis⁸, was added to the incubation medium it did not significantly affect the secretion induced by Br-X537A. The absence of external calcium did not prevent oxytocin secretion by Br-X537A but a sixfold increase in hormone release was observed (from 2.06 ± 0.68 to 11.61 ± 1.36 mU).

These results support the suggestion that external calcium is not essential for hormone release induced by Br-X537A. Furthermore no calcium uptake was observed in the presence of the calcium ionophore in spite of an increased rate of hormone release (Fig. 3a). In fact, an increase in calcium efflux occurs when Br-X537A is added to the medium in which glands preloaded with calcium are incubated. Since this effect is also observed in the absence of external calcium or in a solution lacking sodium and calcium (Fig. 3b), the results cannot be explained by a sodium-calcium exchange previously suggested for the neurohypophysis^{9,10}.

Thus, although it seems that under normal physiological conditions an uptake of external calcium is associated with hormone release, the important step in the stimulation-secretion coupling mechanism seems to be an increase in cytoplasmic ionised calcium concentration. The effect of Br-X537A may therefore be to increase calcium release from an intracellular source, for example, mitochondria, as has already been suggested for the ionophore X537A (ref. 5).

Finally, this new calcium ionophore Br-X537A is very much more potent than the original molecule X537A. Although Br-X537A has not yet been shown to be a calcium ionophore in terms of physicochemical properties, it may be very useful in the study of the biological mechanisms involving calcium, particularly those in which the action of the two previous

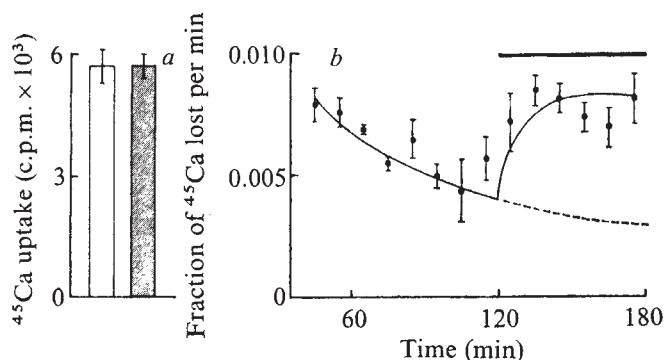


Fig. 3 Effect of Br-537A on ^{45}Ca uptake (a) and efflux (b) from the neurohypophysis. a, After preincubation for 30 min in normal Locke's solution both control and test group of glands were transferred for 10 min to a Na-free solution containing dimethylsulphoxide (1%). The glands were then exposed to the same solution containing radioactive calcium ($7\mu\text{Ci ml}^{-1}$) for 20 min. Ionophore was present at a concentration of $20\mu\text{g ml}^{-1}$ during the last 15 min of exposure to the label. To remove radioactive calcium from the extracellular space both test and control groups of glands were immersed for 2 s in a Na-free solution and then washed for 60 min in a similar solution which was changed every 10 min. The glands were then blotted, weighed, digested overnight in solene and the ^{45}Ca content counted. The total calcium uptake was calculated by adding to the final ^{45}Ca gland content the amount found in the washout samples. Open column: control uptake (mean $\pm$ s.e., $n=5$); hatched column: calcium uptake in the presence of Br-X537A (mean $\pm$ s.e., $n=6$). b, Groups of four glands were loaded for 60 min in a solution containing radioactive calcium ($33\mu\text{Ci ml}^{-1}$). They were then transferred to a solution lacking Na and Ca. After 10 min, the solution was removed, replaced by a fresh solution and counted. This sequence was repeated for up to 240 min. Dimethylsulphoxide (1%) was present throughout all the efflux studies. At the end of the experiment the glands were digested with solene and the ^{45}Ca content counted. Br-X537A ($20\mu\text{g ml}^{-1}$) was added to the perfusate as indicated by the bar. Each point represents the mean rate constant ($\pm$ s.e., $n=4$) of calcium efflux observed during 180 min of washout starting at time 0. The dashed line represents the control experiments.

ionophores described, X537A and A23187, was difficult to observe.

We thank Hoffmann-LaRoche for ionophore. J.J.N. is the recipient of a European Science Exchange Programme Fellowship.

J. J. NORDMANN

Department of Pharmacology,
University of Cambridge,
Cambridge, UK

R. E. J. DYBALL

ARC Institute of Animal Physiology,
Babraham, Cambridge, UK

Received February 24; accepted March 27, 1975.

- Levy, J. V., Cohen, J. A., and Inesi, G., *Nature*, **242**, 461–463 (1973).
- Foreman, J. C., Mongar, J. L., and Compert, B. D., *Nature*, **245**, 249–251 (1973).
- Van Sande, J., and Dumont, J. E., *Biochem. biophys. Res. Commun.*, **62**, 168–175 (1975).
- Nakazato, Y., and Douglas, W. W., *Nature*, **249**, 497–481 (1974).
- Nordmann, J. J., and Currell, G. A., *Nature*, **253**, 646–647 (1975).
- Mikiten, T. M., and Douglas, W. W., *Nature*, **207**, 302 (1965).
- Douglas, W. W., and Poinsner, A. M., *J. Physiol., Lond.*, **172**, 19–30 (1964).
- Dreifuss, J. J., Grau, J. D., and Nordmann, J. J., *J. Physiol., Lond.*, **231**, 96–98P (1973).
- Nordmann, J. J., thesis, Univ. Geneva (1973).
- Dreifuss, J. J., and Nordmann, J. J., *J. Physiol., Lond.*, **240**, 46–47P (1974).
- Bisset, G. W., et al., *Br. J. Pharmacol.*, **31**, 537–549 (1967).
- Neilands, J. B., in *Methods in Enzymology*, 1 (edit. by Colowick, S. P., and Kaplan, N. O.) 449–454 (Academic, New York and London, 1955).

Reappearance of extrajunctional acetylcholine sensitivity in denervated rat muscle after blockage with α -bungarotoxin

In most vertebrate skeletal muscles only the endplate region of muscle membrane is sensitive to acetylcholine (ACh), but after denervation the entire extrajunctional membrane becomes sensitive^{1,2}. In rat diaphragm fibres the degree of extra-

junctional ACh sensitivity increases steadily during the 7–9 d following denervation and then remains relatively constant at a high level for at least several weeks. It has been shown that this increase in ACh sensitivity results from the appearance of new ACh receptors in the extrasynaptic membrane^{3,4}, and an intriguing question is whether in already fully supersensitive fibres new ACh receptors continue to appear in the surface membrane. α -Bungarotoxin (α -BuTX) blocks the depolarising action of ACh on supersensitive muscle membrane⁴, and is thought to bind specifically and almost irreversibly to ACh receptors. Thus one can block all or most receptors of supersensitive muscle fibres at a certain time. I report here experiments in which the reappearance of extrajunctional ACh sensitivity was followed after blocking sensitivity with α -BuTX, using denervated rat diaphragm muscle, maintained in organ culture⁵.

When toxin-blocked muscles were kept at 37 °C ACh sensitivities of fibres tested during the first half hour after transfer to toxin-free medium were below 0.1 mV nC^{-1} , whereas 6 h later extrajunctional ACh sensitivity had already reappeared and was about 5% of the control value (Fig. 1). At 24 h after toxin blockage the mean ACh sensitivity had returned to 60% of the value found in unblocked control fibres of the same diaphragm, and 30–36 h after toxin block there was no statistically significant difference ($P>0.05$) between the sensitivity of fibres exposed to toxin ($317\pm 24\text{ mV nC}^{-1}$; (mean $\pm$ s.e.) 40 fibres) and the sensitivity of fibres kept in normal medium throughout ($346\pm 30\text{ mV nC}^{-1}$; 48 fibres). Resting potentials and input resistances of control fibres and fibres exposed to α -BuTX were similar over the whole period. The time course of reappearance of extrajunctional ACh sensitivity was slower when the muscles were maintained at 27 °C, when full recovery of sensitivity to control values was found only about 70 h after toxin block (Fig. 1). Resting potentials, input

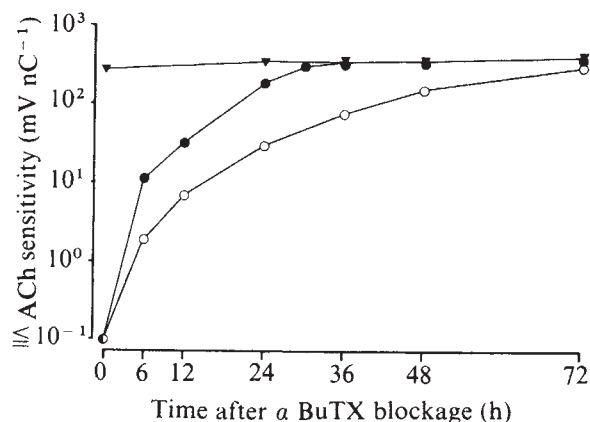


Fig. 1 Reappearance of extrajunctional ACh sensitivity after α -BuTX blockage. ●, Muscles kept at 37 °C; ○, muscles kept at 27 °C. The left hemidiaphragm of adult rats (150–200 g) was removed 9–14 d after denervation, in sterile conditions and cut into six to eight strips which were maintained in culture conditions for periods of up to 5 d (ref. 6). Muscle strips were usually kept for 24 h in normal medium before they were exposed to toxin. Blocking ACh sensitivity of all surface fibres was accomplished by adding α -BuTX ($10\mu\text{g ml}^{-1}$) to the culture medium for 2 h. Muscle strips were then transferred to toxin-free medium to wash out unreacted toxin (zero on time scale). Subsequently the medium in the culture chambers was changed four times every half hour during the following 6 h. Thereafter changes were made at 12 h intervals. Each point represents the mean value of sensitivities found in at least 25 fibres of three different experiments. ▼, Mean sensitivities of control fibres kept in toxin-free medium throughout. Extrajunctional ACh sensitivities were determined immediately after transfer to toxin-free medium, and then at various times after toxin blockage. Sensitivity of single fibres was measured by iontophoretic application of ACh 1–4 mm from the myotendinous junction. Sensitivity measurements were made in oxygenated rat Ringer solution at 27 °C. Pulses of ACh were delivered from pipettes filled with 3 M AChCl solution, and which had resistances of 60–80 M Ω and required a bias current of 5 nA.

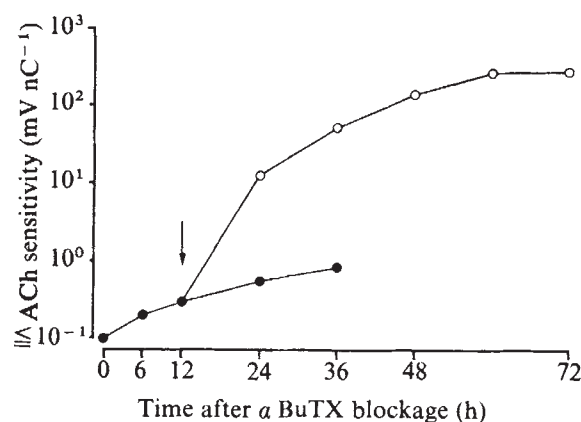


Fig. 2 Effect of cycloheximide ($10 \mu\text{g ml}^{-1}$) on reappearance of extrajunctional ACh sensitivity after α -BuTX blockage. Inhibition of resensitisation in muscles maintained in medium containing cycloheximide 12 h before, during and after toxin blockage (●). When cycloheximide is washed out by transferring muscle strips to cycloheximide-free medium (arrow) and then changing the medium at 1 h intervals for 6 h, ACh sensitivity returns (○). Muscle strips were maintained at 37°C . Each point represents mean values from at least 25 fibres of three different experiments.

resistances and extrajunctional ACh sensitivities of unblocked fibres maintained at 27°C were similar to those found in fibres kept at 37°C .

The relatively fast reappearance of ACh sensitivity after block of receptors with α -BuTX may be caused by dissociation of the toxin-receptor complex. Partial recovery of ACh sensitivity in rat and mouse diaphragm has been reported previously and was attributed to a partly reversibly binding of α -BuTX⁷. Alternatively, return of sensitivity could result from the appearance of new receptors. In this case addition of cycloheximide, an inhibitor of protein synthesis^{8,9} which is known to block the development of denervation supersensitivity^{3,9,10}, should affect resensitisation of toxin-blocked fibres. Therefore, the effect of cycloheximide on the reappearance of ACh sensitivity was determined. Cycloheximide ($10 \mu\text{g ml}^{-1}$) added to the culture medium abolished fibrillatory activity⁶ within 5–7 h of application but had no effect on resting potential, input resistance and ACh sensitivity of unblocked muscle fibres for as long as 48 h. Yet it strongly inhibited resensitisation of toxin-blocked fibres at this concentration. For instance, 36 h after toxin block the degree of resensitisation of muscle fibres kept in medium containing cycloheximide was less than 1% of the value found for resensitisation in cycloheximide-free medium (Fig. 2). If, on the other hand, cycloheximide was washed out from the muscle 12 h after toxin blockage a delayed return of ACh sensitivity to control values was observed (Fig. 2) with a time course slower than that seen in muscles not previously exposed to cycloheximide. Similarly the time course of resensitisation was slowed when muscles were maintained before toxin blockage for 24 h in medium containing cycloheximide ($10 \mu\text{g ml}^{-1}$) which was washed out immediately after toxin blockage. For instance, sensitivity returned to a mean value of $39 \pm 4 \text{ mV nC}^{-1}$ (40 fibres, two experiments) in these muscles 24 h after toxin block whereas in muscle fibres of the same diaphragms kept in cycloheximide-free medium before toxin blockage the corresponding value was $186 \pm 13 \text{ mV nC}^{-1}$ (42 fibres). When muscles were maintained for only 3 h in medium containing cycloheximide before toxin blockage no effect on the time course of resensitisation was seen.

The experiments show that after block of extrajunctional ACh sensitivity with α -BuTX, there is a complete recovery of sensitivity in denervated rat muscle fibres kept in organ culture conditions. Return of sensitivity within a few hours after toxin blockage has been reported for developing chick and rat myotubes¹¹, and this was thought to be caused by the incorporation of newly synthesised ACh receptors into the surface membrane, as blockage of protein synthesis inhibited resensitisation

in these growing fibres. The experiments also show a requirement for protein synthesis for complete resensitisation after toxin blockage in adult muscle fibres. Thus resensitisation in denervated adult muscle fibres could also reflect the appearance of new receptors in the muscle membrane. Whereas in growing myotubes the toxin-receptor complex is stable and additional receptors are incorporated into the membrane¹¹, in adult fibres the new receptors probably replace other receptors which are disappearing. It has recently been shown that α -BuTX, bound to rat diaphragm muscle, is lost^{12,13} and this could reflect degradation of receptor-toxin complex¹².

I thank Dr J. Schmidt for providing α -bungarotoxin.

BERT SAKMANN

Max-Planck-Institut für Biophysikalische Chemie,
D-34 Göttingen, Am Fassberg, West Germany

Received March 26; accepted April 17, 1975.

- 1 Axelson, J., and Thesleff, S., *J. Physiol., Lond.*, **147**, 178–193 (1959).
- 2 Miledi, R., *J. Physiol., Lond.*, **151**, 1–23 (1960).
- 3 Fambrough, D. M., *Science*, **168**, 372–373 (1970).
- 4 Miledi, R., and Potter, L. T., *Nature*, **233**, 599–602 (1971).
- 5 Miledi, R., and Trowell, O. A., *Nature*, **194**, 981–982 (1962).
- 6 Purves, D., and Sakmann, B., *J. Physiol., Lond.*, **237**, 157–182 (1974).
- 7 Lapa, A. J., Albuquerque, E. X., and Daly, J., *Expl Neurol.*, **43**, 375–398 (1974).
- 8 Manchester, K. L., *Nature*, **216**, 394–395 (1967).
- 9 Kimura, M., and Kimura, I., *Nature new Biol.*, **241**, 114–115 (1973).
- 10 Grampp, W., Harris, J. B., and Thesleff, S., *J. Physiol., Lond.*, **221**, 743–754 (1972).
- 11 Hartzell, H. C., and Fambrough, D. M., *Dev Biol.*, **30**, 153–165 (1973).
- 12 Berg, D. K., and Hall, Z. W., *Science*, **184**, 473–475 (1974).
- 13 Chang, C. C., and Huang, M. C., *Nature*, **253**, 643–644 (1975).

Some behavioural signs of morphine withdrawal blocked by conditional stimuli

It has been suggested that the opiate addict gets pleasure both from the physiological effects of the drug and from the complex of environmental stimuli associated with obtaining and administering the drug. These latter stimuli achieve their control over behaviour by a conditioning process and can therefore, in theoretical terms, be considered as conditional stimuli in the Pavlovian sense. Wikler¹ proposes that the repeated occurrence of certain exteroceptive stimuli which accompany the interoceptive stimulation induced by the opiate, results in conditioning of the environment to the drug state.

This theory is receiving support from experimental studies on animals. In rats² and monkeys³, it has been shown that conditional stimuli previously associated with the withdrawal of a drug can elicit physiological disturbances characteristic of withdrawal. In contrast, they may also serve to reverse withdrawal symptoms if previously associated with the injection of the drug. With-

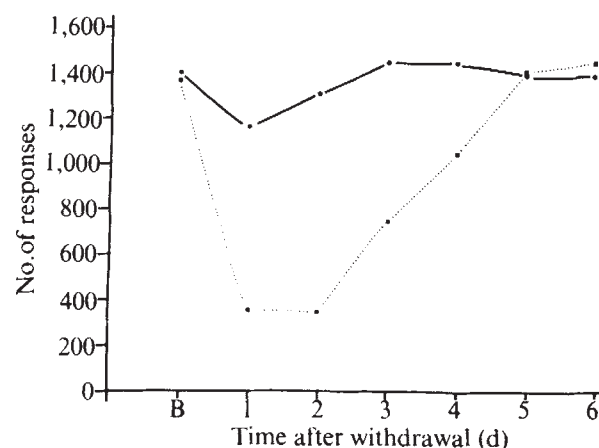


Fig. 1 Responses in VI reinforcement segment during withdrawal for tone + saline (unbroken line) and saline alone (broken line) groups. B, Number of responses on the last day of morphine injection.

Table 1 Mean ($\pm$ s.e.) responses in VI reinforcement during withdrawal

		B	1	2	3	4	5	6
Natural withdrawal	Tone	1,402 (± 79.5)	1,173 (± 80.4)	1,315 (± 63.3)	1,456 (± 70.5)	1,453 (± 68.2)	1,395 (± 82.4)	1,397 (± 82.3)
	No-tone	1,372 (± 79.5)	363 (± 94.1)	357 (± 93.1)	753 (± 92.1)	1,056 (± 164.1)	1,407 (± 133.1)	1,456 (± 168.2)
Naloxone-precipitated withdrawal	Tone	1,482 (± 65.3)	410 (± 92.7)	911 (± 73.6)	1,505 (± 98.4)	1,449 (± 126.0)	1,357 (± 112.6)	1,456 (± 55.6)
	No-tone	1,419 (± 71.4)	296 (± 77.0)	956 (± 92.1)	1,359 (± 64.1)	1,474 (± 55.7)	1,440 (± 87.4)	1,365 (± 98.9)

drawal is characterised by a spectrum of unpleasant physiological effects but it also results in marked disruption of ongoing learned behaviour. We wanted to see if conditional stimuli would prevent the behavioural disruption associated with withdrawal in addition to the reported reversal of physiological symptoms⁴.

Naloxone, which is thought to be a pure morphine antagonist⁵, provides an experimental means of inducing withdrawal. We therefore compared the ability of conditional stimuli to reverse withdrawal symptoms induced by morphine antagonism with those of drug removal. Our results show that auditory stimuli previously paired with the morphine injection prevent the disruption of conditioned behaviour associated with morphine withdrawal but not that associated with naloxone-precipitated withdrawal.

Male Wistar rats (250–350 g) were reduced to 85% free feeding weight and then trained to press a lever for food reward (45 mg Noyes pellets) on a multiple three-component schedule using reward, time-out and a conflict schedule involving reward plus punishment. The three components were signalled by different light conditions—during

days), animals were given intraperitoneal morphine injections twice a day in increasing doses until a maintenance dose was reached. On the first day the injection was 5 mg kg⁻¹ and this was increased by 5 mg kg⁻¹ daily until day 8 when each injection was 40 mg kg⁻¹. Animals were then maintained at this dose, and operant behaviour was tested 30 min after the first daily injection. Injections were administered in a room next to the keeping room. Eight animals were presented with a tone stimulus (12 kHz) during the injection and for 10 min before the test session while a further eight animals received the morphine injection alone. During the second injection the tone presentation was the same but the animals were returned to the home cage after 30 min.

Initially, behaviour in all three segments of the schedule was severely disrupted by morphine. Tolerance to the rate-decreasing effects of the drug gradually developed and after 48 days of testing, animals had returned to pre-drug performance on the three segments of the schedule. Behaviour was then stabilised for a further 10 days. Morphine was then withdrawn and saline injections substituted. A two-way nested analysis of variance was performed on the data. First, considering the variable-interval reinforcement part of the schedule, behaviour was severely disrupted in the saline control group but was not significantly disrupted in animals receiving the tone stimulus plus saline. (Fig. 1 and Tables 1 and 2). Behaviour in the saline group returned to normal limits within 5 d and in both the experimental and saline groups a slight rebound effect was observed during this recovery period. On day 6 of withdrawal morphine injections were resumed after testing and tolerance re-established in both groups under tone and no-tone conditions.

When behaviour had stabilised again, naloxone (0.5 mg kg⁻¹ intraperitoneally) was substituted for morphine to induce withdrawal. The drug was used on day 1 of withdrawal only, after which saline injections were given. A similar analysis of variance was performed on these data. As shown in Fig. 2 and Table 1 behavioural disruption was seen in both groups indicating that conditional stimuli are unable to reverse withdrawal induced by naloxone.

Overall the data indicate that after a sufficient number of pairings with morphine injection a neutral environmental stimulus can evoke behavioural effects resembling those of morphine itself in blocking disruptive withdrawal behavioural phenomena. Responding in the extinction and

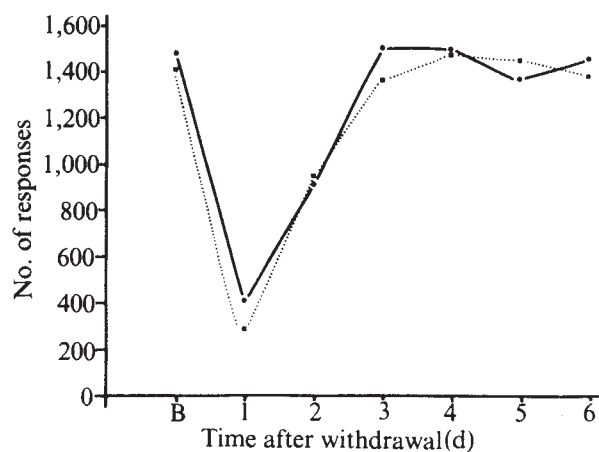


Fig. 2 Responses in VI reinforcement segment during naloxone-precipitated withdrawal for tone+saline (unbroken line) and saline alone (broken line) groups. B, Number of responses on the last day of morphine injection.

reward the illumination was provided by the house light alone; time-out was signalled by darkness; and during conflict both the house light and lever lights were on. This sequence was presented twice during each testing session. Reinforcement in the reward component was available on a variable-interval 25-s (with limits of 5 and 55 s) schedule. No reinforcement was programmed during time-out. During the conflict component, reinforcement was again available on the same variable-interval schedule. Each reinforced response, however, was also punished (0.5 mA electric shock to the feet).

When behaviour had stabilised (the criterion was met when overall session response rates of 6 preceding days did not vary more than 10% from the overall mean for the 6

Table 2 Summary of separate analyses of variance

Factors	Natural withdrawal		Naloxone-precipitated withdrawal	
	F	d.f.	F	d.f.
Groups	16.935	1,14*	0.187	1,14(NS)
(Tone against no-tone)				
Days (days 1–6)	23.723	5,70†	50.799	5,70†
Groups $\times$ days	13.914	5,70†	0.588	5,70(NS)

NS, not significant.

* $P=0.01$; † $P=0.001$.

conflict segments of the schedule was disrupted in a similar manner by morphine withdrawal. The conditional stimuli again prevented this disturbance of ongoing behaviour. Withdrawal induced by naloxone could not, however, be reversed by such conditional stimuli.

Neuropsychologically the result is interesting because it suggests that morphine activates neural pathways associated with the coding of conditional or secondary reinforcing stimuli. Recent studies⁶ on the selective distribution of opiate receptors in the rat brain may provide an important clue for pursuing the neurological basis of this effect.

This work was carried out under the tenure of a SRC grant to N. C. T. On-line control of experiments used the ONLI system developed by C. Crook and S. E. G. Lea with the help of an MRC grant to A. J. Watson. We thank Professor O. L. Zangwill for extending the facilities of the Psychological Laboratory.

N. C. TYE
SUSAN D. IVERSEN

Department of Psychology,
Downing Street,
Cambridge, UK

Received March 12; accepted April 10, 1975.

- ¹ Wikler, A., in *Narcotics* (edit. by Wilner, P., and Kassebaum, G.), (McGraw-Hill, New York, 1965).
- ² Wikler, A., and Pescor, F., *Psychopharmacologia*, **10**, 255-284 (1967).
- ³ Goldberg, S., and Schuster, C., *J. exp. anal. Behav.*, **10**, 235-242 (1967).
- ⁴ Roffman, M., Reddy, C., and Lal, H., *Psychopharmacologia*, **29**, 197-201 (1973).
- ⁵ Drawbaugh, R., and Lal, H., *Nature*, **247**, 65-67 (1974).
- ⁶ Martin, W., *Pharmac. Rev.*, **19**, 463-521 (1967).
- ⁷ Kuhar, M., Pert, C., and Snyder, S., *Nature*, **245**, 447-450 (1973).

Antibody-bound nalorphine released on challenge with morphine

In mice treated with morphine coupled to bovine serum albumin (BSA), antibodies are formed which bind injected morphine and reduce its analgesic effect¹. The possible therapeutic implications of this observation have been speculated on², but a calculation of the number of morphine-binding ligands of a satisfactory antibody titre gives a theoretical binding capacity of only about 15 mg morphine in an adult human. The immunological removal of such low amounts of morphine would be of little practical value in the treatment of high dose intravenous abusers of opiates. One way to improve the effectiveness of the antimorphine principle is to transfer passive immunity³. Another way, which is described here, is the generation of an antibody-bound store of a morphine antagonist which can be released by displacement from the binding sites into an active form when morphine is injected.

In preliminary experiments female NMRI mice (body weight at end of experiment 27 ± 0.5 g) were pretreated with BSA-morphine once a week for 6 months as described by Berkowitz and Spector¹. It was found, however, when the antiserum properties were tested *in vitro*, that to achieve a 50% displacement of ³H-dihydromorphine binding, about 2×10^5 times more nalorphine was needed compared with morphine (Fig. 1a). Thus the antibody formed by BSA-morphine treatment was rather specific for morphine. For that reason a corresponding long term treatment with BSA-conjugated nalorphine was undertaken in another group of mice.

Synthesis of carboxymethyl nalorphine was carried out according to Miller *et al.*⁴, by reacting β -chloroacetic acid with nalorphine hydrochloride containing a trace of ³H-nalorphine. The product was purified on an anion-exchange resin (Dowex 1X4, 200-400 mesh, 13 × 1 cm). The carboxymethyl nalorphine formed was homogenous on paper electrophoresis at pH 6.5. Conjugation of carboxymethyl nalorphine to BSA essentially followed the method of Spector and Parker⁵, except that, instead of the dialysis step, the

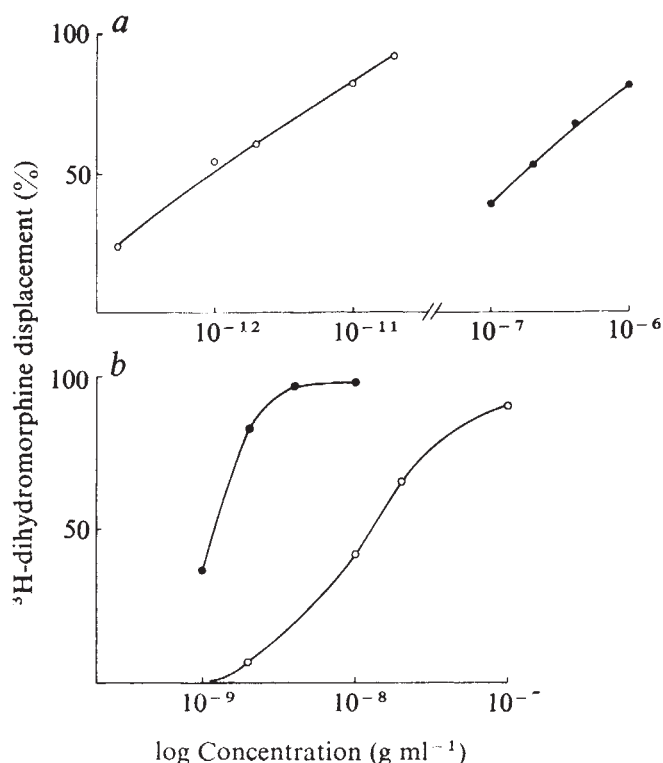


Fig. 1 a, Specificity of antiserum to BSA-morphine. The antiserum exhibits about 2×10^5 -fold greater affinity for morphine (○) than for nalorphine (●). b, Specificity of antiserum to BSA-nalorphine. The antiserum had about ten times more affinity for nalorphine than for morphine.

conjugate was isolated by exclusion chromatography on a Sephadex (G-25 fine) column (30 × 2.5 cm). Analysis of the conjugate for radioactivity and protein showed that four or five molecules of carboxymethyl nalorphine had reacted with each BSA molecule. Each experimental animal received subcutaneously 50 μ l of a solution containing Freund's adjuvant + 1 μ g BSA-nalorphine once a week for 6 months, and control animals were given corresponding amounts of saline or adjuvant (buffered pH 7.5) during the same time period.

The properties of the antiserum obtained from mice treated with BSA-nalorphine was studied in a dilution of 1 : 16 using 0.31 μ Ci ³H-dihydromorphine (specific activity 51.3 Ci mol⁻¹; New England Nuclear) in the presence of morphine or nalorphine⁶. A 50% inhibition of ³H-dihydromorphine binding to the antibodies required about ten times

Table 1 Percentage increase in pain thresholds.

Treatment once a week for 6 months	Treatment 26 h before zero time	Treatment at zero time	Percentage increase in pain threshold* (0-150 min)
Saline (n=10)	Saline	Morphine	266 ± 25
	Nalorphine	Morphine	265 ± 22†
Freund's adjuvant (n=10)	Saline	Morphine	229 ± 18
	Nalorphine	Morphine	215 ± 13†
BSA-nalorphine (n=9)	Saline	Morphine	245 ± 18
	Nalorphine	Morphine	114 ± 7‡

* Means ± s.e. of measurements at nine time points.

† Difference from animals receiving saline 26 h earlier not significant.

‡ Difference from animals receiving saline 26 h earlier; $P < 0.001$. Significance tested by analysis of variance, for the whole time course.

more morphine than nalorphine (Fig. 1b), indicating a relative specificity for nalorphine in this antiserum.

To test the *in vivo* effect on the possible immunopharmacological antagonism of morphine-induced analgesia, nociceptive stimulation was carried out^{6,7}. Using a standardised electrical stimulation of the tail of the animals, the changes in the threshold for vocalisation were registered. Before administration of a drug the individual (normal) threshold was determined and after injection at regular intervals for 150 min. The graded response was expressed as percentage increase in the individual (normal) pain thresholds.

The response to morphine was tested in the three groups of mice treated chronically with saline, adjuvant or BSA-nalorphine. Twenty-six hours before the morphine injection (15 mg kg^{-1} subcutaneously), nalorphine (5 mg kg^{-1} subcutaneously) or saline was given to these three groups of animals. Table 1 shows that only in mice pretreated with BSA-nalorphine + nalorphine a significant reduction in the analgesic response occurred.

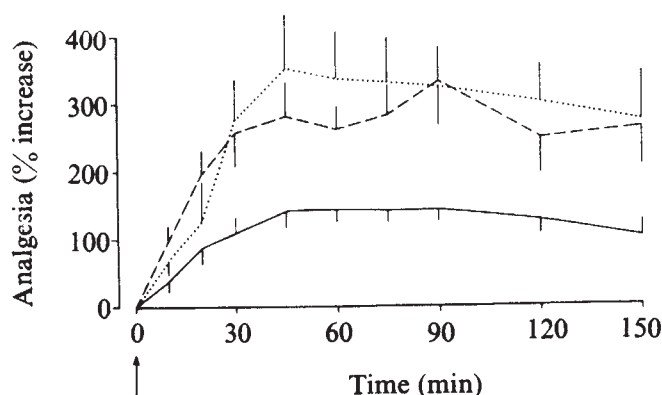


Fig. 2 Percentage increase ($\pm$ s.e.) in threshold for vocalisation after saline chronically and subcutaneous administration of nalorphine (5 mg kg^{-1}) 26 h before (dotted line); BSA-nalorphine chronically and saline 26 h before (dashed line); or BSA-nalorphine chronically and nalorphine 26 h before (solid line) morphine (15 mg kg^{-1}) at arrow.

Figure 2 shows the time curves for analgesia in control mice given nalorphine 26 h before, in animals given BSA-nalorphine chronically and saline 26 h before, and in animals given BSA-nalorphine chronically and nalorphine 26 h before the morphine injection. The last combination gave a significant reduction in analgesia, indicating that the nalorphine injection 26 h before had a considerably prolonged antimorphine action. The antimorphine effect of an even higher dose of nalorphine (10 mg kg^{-1}) in morphine-dependent mice has been reported to last only 1.5–2 h (ref. 8).

Our results thus demonstrate a new immunopharmacological approach—prolonged binding of a short-acting morphine antagonist to a circulating pool of antibodies, from which it can be released by injection of morphine.

This study was supported by a grant from the Tri-centennial Fund of the Bank of Sweden and S. Thånell's Memorial Fund. We thank Pharmacia Ltd for samples of BSA-morphine.

LARS-M. GUNNE
JOHN JONSSON
LENNART PAALZOW
ERIK ÄNGGÅRD

Psychiatric Research Center,
Ulleråker Hospital, 750 17 Uppsala, and
Department of Pharmacology,
University of Uppsala, 751 23 Uppsala, Sweden

Received March 3; accepted April 7, 1975.

- Berkowitz, B., and Spector, S., *Science*, **178**, 1290–1292 (1972).
- Szara, S., *Narcotic Antagonists in Treating Opiate Dependence* (Ninth Congr. Coll. Int. Neuropsychopharmac., in the press).
- Berkowitz, S., Ceretta, K. V., and Spector, S., *Life Sci.*, **15**, 1017–1028 (1974).
- Miller, C., Leung, C. Y., Nakamura, J., Winters, W. D., and Benjamini, E., *Proc. West. Pharmac. Soc.*, **16**, 25–28 (1973).
- Spector, S., and Parker, C. W., *Science*, **168**, 1347–1348 (1970).
- Paalzow, L., *Acta Pharm. Suecia*, **6**, 193–206 (1969).
- Paalzow, L., *Acta Pharm. Suecia*, **6**, 207–226 (1969).
- Huidobro, F., and Maggiolo, C., *Acta Physiol. Lat. Am.*, **11**, 201–209 (1961).

Lead-induced inhibition of brain adenylyl cyclase

ACUTE lead toxicity can produce such neurological signs as ataxia, peripheral neuropathy, seizures, coma and even death¹. Chronic low level exposure to lead, however, may be accompanied by more subtle defects in behaviour, such as hyperactivity, aggressiveness and shortened attention span^{2–4}. Although lead can cause small (15–25%) and variable changes in the level of brain catecholamines^{3,5,6}, no biochemical basis has been clearly established for these behavioural defects. We have now found, however, that very low concentrations of lead inhibit adenylyl cyclase activity in mammalian brain tissue.

The basis for our investigation is the recent evidence that the effects of certain neurotransmitters may be mediated by adenosine 3',5'-monophosphate (cyclic AMP), which is formed in neurones through the activation of the membrane-bound enzyme, adenylyl cyclase⁷. In the mammalian caudate nucleus, for example, a dopamine-sensitive adenylyl cyclase has been identified with properties quite similar to those of the caudate dopamine receptor⁸. Furthermore, in the cerebellum, a noradrenaline-sensitive adenylyl cyclase seems to mediate the inhibitory effect of noradrenaline on Purkinje cells⁹. Because both dopamine and noradrenaline are known to affect behaviour, we investigated the possibility that the behavioural deficits induced by toxic lead exposure may be the result of an alteration of cyclic AMP metabolism.

We examined the adenylyl cyclase of rat cerebellum because the action of cyclic AMP in this tissue has been well studied both biochemically and electrophysiologically^{9,10}. Figure 1 shows the effect of lead nitrate on the rate of formation of cyclic AMP from ATP in cerebellar homogenates. Enzyme activity was inhibited by concentrations of lead as low as $0.1 \mu\text{M}$: 50% inhibition occurred at about $3 \mu\text{M}$ and almost complete inhibition at $100 \mu\text{M}$. Lead chloride had the same effect. As well as affecting basal adenylyl cyclase activity, lead ions also inhibited noradrenaline-stimulated activity. In contrast to the effect on cyclic AMP formation, similar concentrations of lead had little effect on the rate of hydrolysis of cyclic AMP, indicating that in the experimental conditions the observed decrease of cyclic AMP formation was the result of inhibition of adenylyl cyclase and not of stimulation of phosphodiesterase.

The inhibitory effect of lead was found to be independent of

Table 1 Effect of washing and 2-mercaptoethanol on lead-inhibited adenylyl cyclase activity in particulate fractions from rat cerebellum

	Activity (pmol per mg protein per min)
Control	90 ± 6
+ PbCl_2 ($100 \mu\text{M}$)	0 ± 2
+ 2-mercaptoethanol (2 mM)	186 ± 14
+ PbCl_2 + 2-mercaptoethanol	29 ± 5
+ PbCl_2 * + washing	32 ± 2
+ PbCl_2 * + washing + 2-mercaptoethanol	75 ± 2

Adenylyl cyclase activity was determined as in Fig. 1, except that the source of enzyme was a particulate fraction obtained by centrifuging the crude homogenate at $30,000g$ for 30 min, suspending the pellet in 6 mM Tris-maleate buffer, and recentrifuging and washing twice more.

* In some cases the homogenate was preincubated with lead ($100 \mu\text{M}$) for 10 min at 30°C before centrifugation and washing.

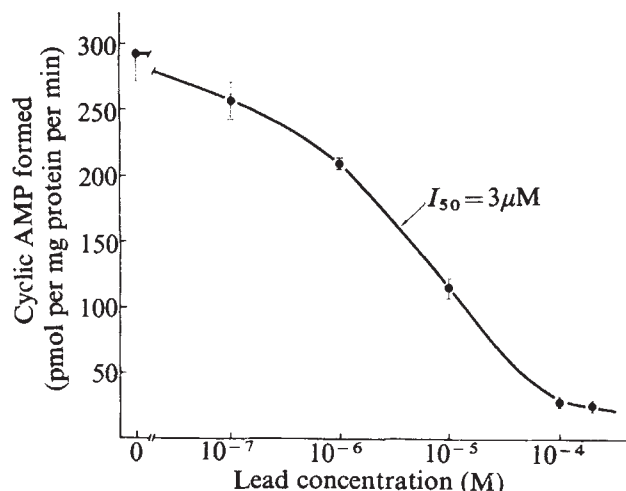


Fig. 1 Effect of various concentrations of lead nitrate on the adenylyl cyclase activity of a rat cerebellar homogenate. The values shown here and in Table 1 are the means and ranges for two to four replicate samples, each assayed for cyclic AMP content in duplicate. To measure enzyme activity, 1 mm thick slices of cerebellar cortex from adult, male, Sprague-Dawley rats were homogenised (15 mg ml⁻¹) in 6 mM Tris-maleate buffer (pH 7.4). The rate of cyclic AMP formation was measured in an assay system containing 80 mM Tris-maleate (pH 7.4), 10 mM theophylline, 6 mM MgSO₄, 1.5 mM ATP, and tissue homogenate (1 mg wet weight) plus or minus lead salt or other test substances, in a final volume of 0.3 ml. Incubation was for 3 min at 30 °C in a shaken water bath. The reaction was initiated by addition of ATP, terminated by boiling for 2 min, and then centrifuged at low speed to remove insoluble material. Cyclic AMP in the supernatant was measured by the method of Brown, *et al.*²². In the experimental conditions used, enzyme activity was linear with respect to time and enzyme (protein) concentration. At concentrations of lead greater than 500 μM, the amount of cyclic AMP present in the reaction mixture increased. Much of this increase was found to be caused by a non-enzymatic conversion of ATP to cyclic AMP.

the concentration of ATP used, making it unlikely that enzyme inhibition was simply caused by a decrease in the concentration of available substrate (which might have occurred because of the known non-enzymatic hydrolysis of ATP by lead¹¹). The inhibitory effect of lead was also found to be independent of the tissue protein concentration present in the reaction mixture.

In other experiments in which washed particulate fractions were used as a source of enzyme (Table 1), the inhibitory effect of lead was similar to that found in homogenates. Furthermore, tissue preparations which had first been exposed to lead ions and then extensively washed in lead-free buffer showed only a 30% recovery of enzyme activity. We also found that treatment of particulate fractions with the sulphhydryl-reducing agent, 2-mercaptoethanol, increased control activity (an effect described previously¹²) but had little effect on lead-inhibited activity. Moreover, after exposure to lead, a combination of washing and treatment with 2-mercaptoethanol only partially reactivated enzyme activity, suggesting that the inhibitory effect of lead was largely irreversible in these conditions.

The concentration of lead in the brain tissue of rats exposed to a normal diet varies from 0.5 to 1.0 μM (refs 3, 13). At this level, in our study, adenylyl cyclase activity was inhibited 10–20%. In cases of experimental lead intoxication in which rats display symptoms of hyperactivity, brain lead levels vary from 3.0 to 6.0 μM (ref. 3) a concentration at which, in our study, adenylyl cyclase activity was inhibited 50–70%. Future studies will be required to determine whether animals made hyperactive on lead-containing diets show inhibition of adenylyl cyclase activity *in vivo*.

Several reports have suggested that cyclic AMP may be involved in the facilitation of neuromuscular transmission

either in normal conditions or during neuromuscular fatigue^{14,15}. It is known, also, that lead ion can block neuromuscular transmission, the effect being more pronounced after long periods of stimulation^{16,18}. Thus, the inhibition by lead of adenylyl cyclase activity offers one possible explanation for the inhibitory effects of lead on neuromuscular transmission. It is also known that blockade of adrenergic receptors can induce reciprocal changes in catecholamine turnover^{19,20}. Since inhibition of adenylyl cyclase may be equivalent to receptor blockade, it is possible that the changes in catecholamine levels reported in lead-treated animals^{3,5,6} could be related to the inhibitory effect of lead on adenylyl cyclase *in vivo*.

Because lead influences the activity of several enzymes²¹, the biochemical basis of lead toxicity in the nervous system is likely to be complex. Nevertheless, the marked inhibitory effect of low concentrations of lead on an enzyme thought to be intimately involved in synaptic transmission suggests that interference with cyclic AMP metabolism by this ion could be a factor in some of the neurological manifestations of lead toxicity.

Note added in proof: After this manuscript was accepted, LeMay and Jarett²³ reported that lead inhibits the adenylyl cyclase activity of fat cell membranes and pancreatic islet homogenates in conditions used for histochemical localisation of enzyme activity. Thus, our suggestion that inhibition of adenylyl cyclase may mediate some of the toxic manifestations of lead in the nervous system may also have implications for other organ systems.

JAMES A. NATHANSON
FLOYD E. BLOOM

Laboratory of Neuropharmacology,
National Institute of Mental Health,
St Elizabeths Hospital,
Washington DC 20032

Received February 21; accepted April 21, 1975.

- Goyer, R. A., and Rhyne, B. C., *Int. Rev. exp. Path.*, **12**, 1–77 (1972).
- David, C., Clark, J., and Voeller, K., *Lancet*, **ii**, 900–903 (1972).
- Sauerhoff, M. W., and Michaelson, I. A., *Science*, **182**, 1022–1024 (1973).
- Silbergeld, E. K., and Goldberg, A. M., *Life Sci.*, **13**, 1275–1283 (1973).
- Michaelson, I. A., Greenland, R. D., and Roth, W., *Pharmacologist*, **16**, 250 (1974).
- Silbergeld, E. K., and Goldberg, A. M., *Pharmacologist*, **16**, 249 (1974).
- Greengard, P., Nathanson, J. A., and Kebabian, J. W., in *Frontiers in catecholamine research* (edit. by Usdin, E., and Snyder, S.), 377–382 (Pergamon, New York and London, 1973).
- Kebabian, J. W., Petzold, G. L., and Greengard, P., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2145–2149 (1972).
- Hoffer, B. J., Siggins, G. R., Oliver, G. L., and Bloom, F. E., *Adv. cyclic Nucleotide Res.*, **1**, 411–423 (1972).
- Klainer, L. M., Chi, Y.-M., Friedberg, S. L., Rall, T. W., and Sutherland, E. W., *J. biol. Chem.*, **237**, 1239–1243 (1962).
- Rosenthal, A. S., Moses, H. L., Beaver, D. L., and Schuffman, S. S., *J. histochem. Cytochem.*, **14**, 698–701 (1966).
- Johnson, R. A., and Sutherland, E. W., *J. biol. Chem.*, **248**, 5114–5121 (1973).
- Krigman, M. R., *et al.*, *J. Neuropharmacol. exp. Neurol.*, **33**, 58–73 (1973).
- Goldberg, A. L., and Singer, J. J., *Proc. natn. Acad. Sci. U.S.A.*, **64**, 134–141 (1969).
- Miyamoto, M. D., and Breckenridge, B. M., *J. gen. Physiol.*, **63**, 609–624 (1974).
- Manalis, R. S., and Cooper, G. P., *Nature*, **243**, 354–356 (1973).
- Silbergeld, E. K., Fales, J. T., Goldberg, A. M., *Nature*, **247**, 49–50 (1974).
- Silbergeld, E. K., Fales, J. T., and Goldberg, A. M., *Neuropharmacology*, **13**, 795–801 (1974).
- Nyback, H., Wiesel, F.-A., and Sedvall, G., in *Frontiers in catecholamine research* (edit. by Usdin, E., and Snyder, S.), 601–604 (Pergamon, New York and London, 1973).
- Dairman, W., Gordon, R., Spector, S., Sjoerdsma, A., and Udenfriend, S., *Molec. Pharmacol.*, **4**, 457–464 (1968).
- Vallee, B. L., and Ulmer, D. C., *A. Rev. Biochem.*, **41**, 91–128 (1972).
- Brown, B. L., Elkins, R. P., and Albano, J. D. M., *Adv. cyclic Nucleotide Res.*, **2**, 25 (1972).
- LeMay, and Jarett, *J. Cell Biol.*, **65**, 39–50 (1975).

***In vitro* conversion of a calf thymus 8S DNA polymerase to a 7.3S species**

MULTIPLE species of calf thymus high molecular weight (6–8S) DNA polymerase have been distinguished by DEAE-cellulose chromatography and partially characterised^{1–4}. In order of elution these were designated enzymes A(8S), B(5.2S) and C(7.3S), and preliminary estimations indicated molecular weights of 200×10^3 – 230×10^3 , 100×10^3 – 110×10^3 and 155×10^3 – 175×10^3 respectively¹. Subsequent experiments have resolved the A enzyme into two components, A₁ and A₂, eluting

between 55 and 75 mM and 75 and 95 mM potassium phosphate pH 7.8, respectively (Fig. 1 and ref. 2). The relative levels of activity of A₁, A₂, B and C vary considerably in different preparations and we show here that on urea treatment, A₂ (sedimenting at around 8S), is converted into a species sedimenting at 7.3S, the sedimentation coefficient of the C species.

A₁ and A₂ show similar properties; both elute from Sepharose 6B in 0.5 M NaCl, 0.05 M Tris HCl pH 8.5 (buffer I) at an elution volume (*V*_e) approximately 1.68 times the void volume (*V*₀), and both sediment on 10–30% glycerol gradients, made in buffer I, at 7.9–8.1S. When the NaCl in these gradients is reduced to 0.05 M, both A₁ and A₂ sediment with a main peak at 10.5–10.7S and a shoulder at 8.4–8.7S (ref. 2). Since the 280 nm:260 nm absorbance ratios for A₁ and A₂ were 1.6–1.7, nucleic acid is unlikely to account for the separation between them. One further feature of the DEAE-cellulose profile is the poly(dA)-oligo(dT)₁₀ preferring activity, designated enzyme D (ref. 1) which elutes between 105 and 125 mM potassium phosphate; it sediments on glycerol gradients made in buffer I between 6.6–7.1S. Terminal transferase and low molecular weight DNA polymerase activities were not detectable having been removed by a previous step on Sepharose 6B.

The levels of activity in peaks A₁, A₂, B and C have been found to vary in an unpredictable way in different preparations. For example in Fig. 1, the amount of 5.2S enzyme, eluting between 95 and 110 mM phosphate, is considerably reduced compared with that seen previously¹ although glycerol gradient analysis clearly reveals its presence. The proportion of enzyme C has varied between being the major and a minor component.

To explore the basis of this variability, A₁ and A₂ regions were separately pooled, concentrated and rechromatographed on DEAE-cellulose, when A₁ again eluted between 55 and 75 mM phosphate with a shoulder of A₂, and A₂ eluted mainly between 75 and 95 mM phosphate. In both cases a small amount of activity (10–20% of the total in different experiments) eluted between 125 and 160 mM phosphate in the

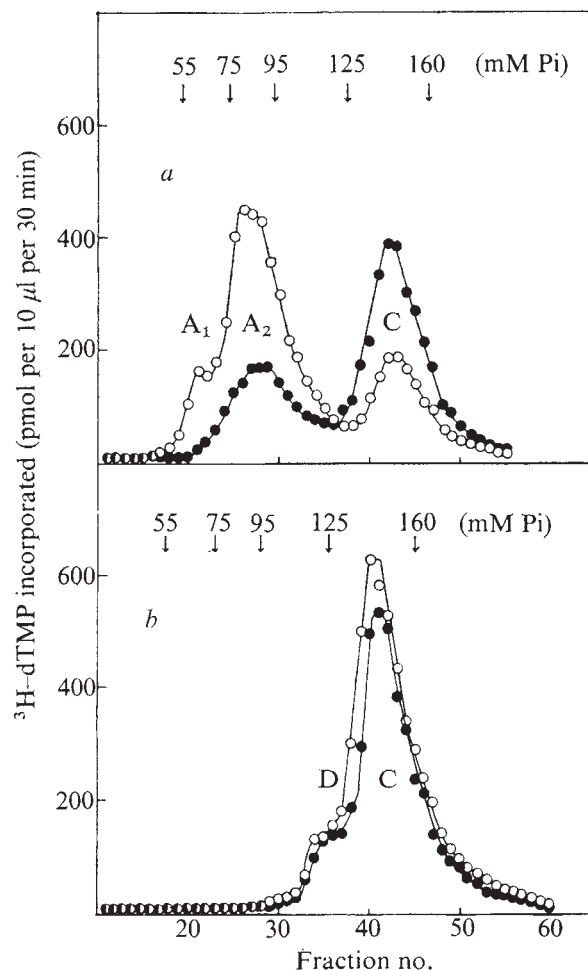
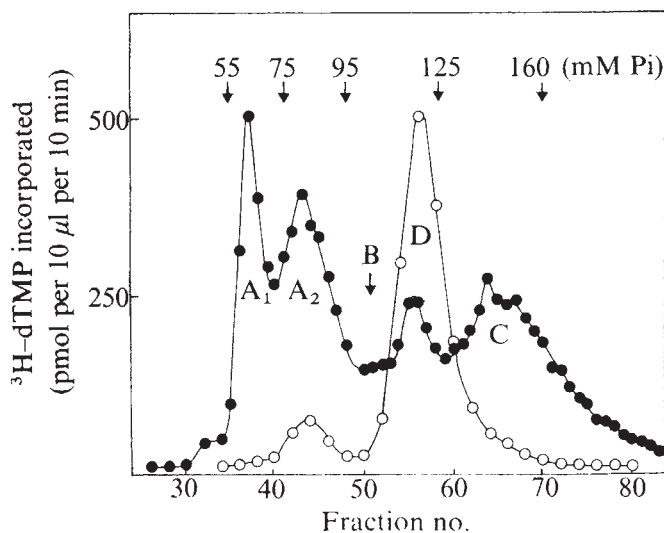


Fig. 2 Rechromatography of calf thymus high molecular weight DNA polymerase with and without previous urea treatment. DNA polymerases A₂ and C were separately pooled from a DEAE-cellulose run like that shown in Fig. 1 and samples were rechromatographed on a 16×1 cm DEAE-cellulose column immediately after preincubation at 0 °C with or without 2.4 M urea (protein concentration 0.5–1 mg ml⁻¹ in 0.03 M phosphate pH 7.8). Elution was with a 150-ml linear gradient from 0.03 to 0.25 M potassium phosphate pH 7.8; fractions were 2.5 ml. *a*, 3,100 units, 17 mg, enzyme A₂; *b*, 3,000 units, 8.5 mg enzyme C were used in each run with urea (●) or without urea (○).

Fig. 1 DEAE cellulose chromatography of the calf thymus high molecular weight DNA polymerase fraction. 84,000 units, 220 mg of DNA polymerase fraction IV enzyme¹ were applied to a 50×1.6 cm DEAE-cellulose column in 0.03 M potassium phosphate pH 7.8 and then eluted with a 1,000-ml linear gradient up to 0.25 M potassium phosphate, pH 7.8; fractions, 12 ml. DNA polymerase assays using either activated calf thymus DNA or poly(dA)-oligo(dT)₁₀ (20:1 base ratio) were carried out as described¹. Terminal transferase was assayed using (dA)₄ and a Mg²⁺-dATP assay⁵. One unit of enzyme activity is defined as the incorporation of 1 nmol ³H-dTMP in 60 min. All buffers contained 20% glycerol and 1 mM dithiothreitol. Phosphate concentrations in gradient fractions were determined at 22 °C using a Radiometer Conductivity meter (type CDM 2d). ●, Activated DNA; ○, poly(dA)-oligo(dT)₁₀, assays.



region of enzyme C (7.3S). This was too far removed from the A enzymes to be a simple contaminant. On preincubating A₂ at 0 °C with 2.4 M urea (a concentration which caused only slight loss of enzyme activity), the activity eluting between 125 and 160 mM phosphate increased substantially (Fig. 2*a*). Recovery of activity from columns was 70–85% of that loaded. Enzyme remaining in the A position still sedimented typically at 8–8.5S; on further exposure to 2.4 M urea 50–60% of total polymerase activity again appeared in the C region on DEAE-cellulose. If preincubation with 2.4 M urea was followed by 60 min in 2.8 M urea, complete conversion of A₂ occurred. Results with A₁ were identical to those obtained with A₂.

The enzymes derived from both A₁ and A₂ by the action of urea were pooled and found to resemble enzyme C in that they sediment on 10–30% glycerol gradients in buffer I at 7.3–7.4S (ref. 1), they elute from Sepharose 6B at *V*_e/*V*₀ 1.80–1.85 (ref. 1) and they elute from DEAE-cellulose between 125 and 160 mM phosphate^{1,2}. In none of our experiments has urea treatment of A or C enzymes resulted in the formation of the 5.2S enzyme (Fig. 2*b*) and although it resembles the A (8S) and C (7.3S) enzymes in several respects¹, its origin remains obscure. The elution position of enzyme D seems unaffected by 2.4–2.8 M urea treatment.

To some extent, therefore, the variability in the DEAE-

cellulose elution profile can be explained by the fact that enzyme C derives from A_1 and A_2 . Although the elution positions of the derived C enzymes seem to be the same on DEAE-cellulose, a slight difference in charge properties may exist and could account for the double peak in the C region of Fig. 1. In either case urea seems to remove a basic subunit of molecular weight about 50,000–70,000. Attempts to recombine C enzyme with DEAE-cellulose flow-through material, further fractionated on CM-cellulose, have not yet been successful.

This work was supported by a MRC research grant to I.R.J. and a MRC Research Studentship to I.P.H. A.M.H. is a Beit Research Fellow. We thank W. F. Wakeling for technical assistance.

A. M. HOLMES
I. P. HESSLEWOOD
I. R. JOHNSTON

Department of Biochemistry,
University College London,
Gower Street, London WC1E 6BT, UK

Received March 17; accepted April 22, 1975.

¹ Holmes, A. M., Hesselwood, I. P., and Johnston, I. R., *Eur. J. Biochem.*, **43**, 487–499 (1974).

² Holmes, A. M., Hesselwood, I. P., and Johnston, I. R., *Trans. Biochem. Soc.*, **2**, 864–865 (1974).

³ Momparler, R. L., Rossi, M., and Labitan, A., *J. Biol. Chem.*, **248**, 285–293 (1973).

⁴ Yoshida, S., Kondo, T., and Ando, T., *Biochim. biophys. Acta*, **353**, 463–474 (1974).

⁵ Chang, L. M. S., and Bollum, F. J., *J. Biol. Chem.*, **246**, 909–916 (1971).

More similarity between bakers' yeast L-(+)-lactate dehydrogenase and liver microsomal cytochrome b_5

BAKERS' yeast L-lactate dehydrogenase, or cytochrome b_2 (EC 1.1.2.3.), catalyses the oxidation of L-(+)-lactate to pyruvate. When purified in the presence of phenylmethanesulphonylfluoride, it is a tetramer of four presumably identical polypeptide chains of molecular weight 57,000, each of which carries one flavin mononucleotide and one protohaem IX (ref. 1). In the absence of protease inhibitor, the enzyme obtained by the classical crystallisation method

of Appleby and Morton² shows cleavages at the N terminus and at a region about two-thirds down the chain, in each monomer; it is then composed of four chains (α) of molecular weight about 36,000 and four chains (β) of about 21,000 (ref. 1).

Yeast proteases present in the autolysate can further degrade the cleaved enzyme; the final product is a flavin-free haem-binding fragment (cytochrome b_2 core) of molecular weight 11,000³; trypsin digestion yields the same ultimate fragment⁴. Cytochrome b_2 core has been studied intensively as a simplified model of the haem-binding site in the flavohaemoprotein. Absorption spectroscopy^{3,4}, electron paramagnetic resonance⁵ and nuclear magnetic resonance⁶ studies, as well as photooxidation experiments⁷, showed that the haem environment in cytochrome b_2 core (and thus in cytochrome b_2 itself) is very similar to that in liver microsomal cytochrome b_5 . Recently completed work in this laboratory showed a marked sequence similarity between the two proteins⁸. Taken together, all these data suggest that cytochrome b_2 core has an overall three-dimensional folding very similar to that of cytochrome b_5 , and that the two proteins are evolutionarily related⁸.

The above findings have raised the question of the integration of the 11,000-dalton fragment in the molecular edifice of native cytochrome b_2 . We carried out automatic degradation experiments on the N terminus of the intact cytochrome b_2 core subunit, and on the α chain of the cleaved enzyme. The results (Table 1) clearly indicate that cytochrome b_2 core sequence begins exactly at the N terminus of the latter, and that the yeast proteases remove the seven preceding residues when they cleave the intact enzyme during purification in the absence of inhibitor. It had already been shown that the core is part of the α chain^{9,10}; our data enable a more precise determination of its location in the linear sequence, and extend the scheme presented in a previous paper¹ (Fig. 1).

It has been suggested before that the cytochrome b_2 monomer can be folded into at least two globular domains, encompassing the haem and flavin-binding sites, respectively (they could correspond to the α and β subunits); these domains may have become covalently bonded as a result of a gene fusion process^{1,11}. It is clear that the cytochrome b_2 core region can constitute a structural domain in itself (ref.

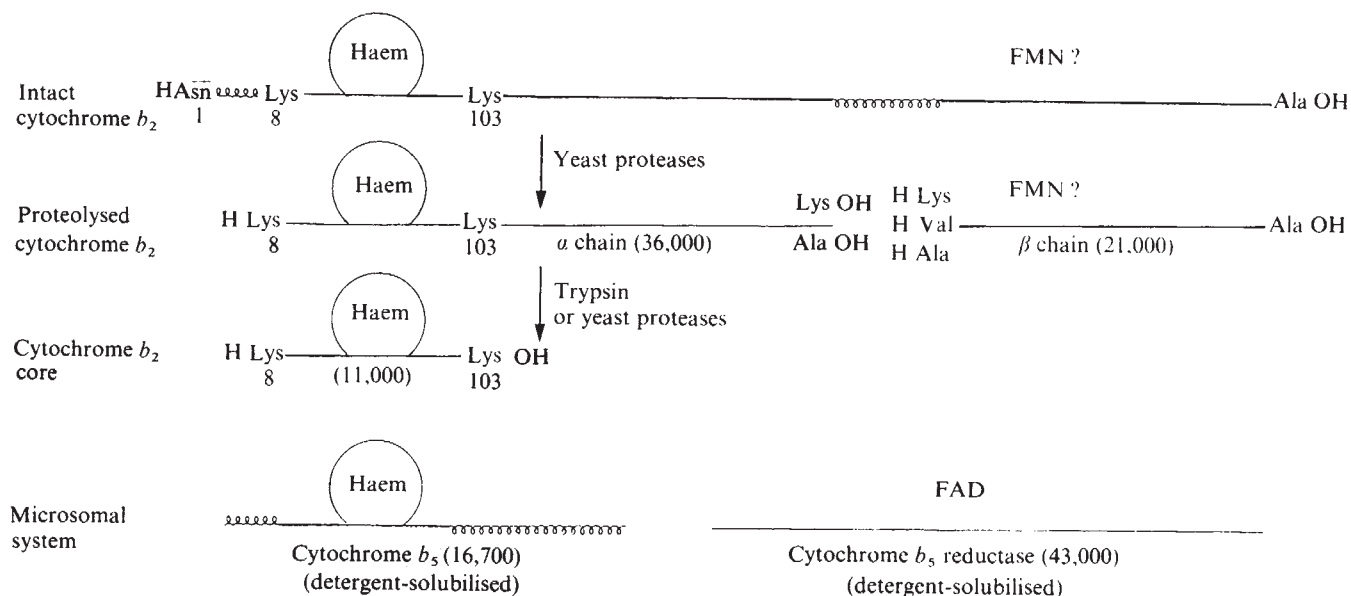


Fig. 1 Linear representation of cytochrome b_2 and cytochrome b_5 -cytochrome b_5 reductase system. Spirals, fragments released by proteases. Cytochrome b_5 reductase solubilised by lysosomal action has a molecular weight of about 32,000; the localisation of the lost hydrophobic fragment (at the N or C terminus) is undefined at present^{21,22}. FMN, Flavin mononucleotide; FAD, Flavin adenine dinucleotide.

Table 1 N-terminal sequences of various cytochrome *b*₂ derivatives and detergent-solubilised cytochrome *b*₂

Intact b_2^*

H-Asn-Pro-Lys-Leu-Asp-Met-Asn-Lys-Gln-Lys-Ile-Ser-Pro-Ala-

1 5 10

α chain*

H-Lys-Gln-Lys-Ile-Ser-Pro-Ala-Glu-Val-Ala-Lys-His-Asn-

10 15 20

b_2 core†

H-Lys-Gln-Lys-Ile-Ser-Pro-Ala-Glu-Val-Ala-Lys-His-Asn-Lys-Pro-...

10 15 20

Human $b_{\beta}^\ddagger$

Z(Asx,Glx)-Glu-Glu-Ala-Ser-Asp-Glu-Ala-Val-Lys-Tyr-Tyr-Thr-Leu-Glu-Glu-Ile-Glu-Lys-His-Asn-His-Ser-...

Bovine $b_{\beta}^\S$

Z(Asx,Glx,Glx,Glx,Ala,Ser)-Ser-Lys-Ala-Val-Lys-Tyr-Tyr-Thr-Leu-Glu-Gln-Ile-Glu-Lys-His-Asn-Asn-Ser-...

Pig $b_{\beta}^\ddagger$

Z-Glx-Glx-Asp-Ala-Ser-Lys-Ala-Val-Lys-Tyr-Tyr-Thr-Leu-Gln-Glu-Ile-Glx-Lys-His-Asn-Asn-Ser-...

The vertical arrows represent the points of specific proteolytic cleavage during preparation of the proteins. Z, absence of free terminal amino group.

*Determined by automated Edman degradation with a Socosi sequenator PS100, according to published procedures⁸, using 0.08 μmol untreated enzyme and 0.13 μmol reduced S-carboxymethylated α chain. The former was purified according to ref. 1, the latter according to ref. 18.

†Ref. 8 and Guiard and Lederer (unpublished).

‡The sequences before and after the arrows are reproduced from Ozols^{12,19} and from ref. 20, respectively.

8 and B. G. and F. L., unpublished), although we do not know how independent a structural unit it represents in the intact molecule. The fact that it is situated at the N terminus of the protein makes one wonder about its possible importance in the folding of the peptide chain during biosynthesis.

Of particular interest is the comparison with results obtained by Ozols¹² on liver cytochrome b_5 from various species. Solubilisation of that protein with detergents leads to a longer molecule than solubilisation with proteases^{13,14}. In addition to a hydrophobic tail on the C terminal side, which is responsible for the attachment to the membrane^{15,16}, detergent b_5 has a few more amino acids on the N terminal side¹². The partial sequences are shown in Table 1 in such a way as to respect the alignment presented elsewhere⁸ between cytochrome b_2 core and proteolysed cytochrome b_5 . It becomes clear (see also Fig. 1) that the structural similarity between the two fragments⁸ extends to the N terminal regions of the undigested systems. These may be rather flexible, as even the first two residues of lipase-solubilised calf liver cytochrome b_5 are disordered in the crystal¹⁷. This flexibility could explain the easy cleavage by proteases.

The experiments reported here, in our view, strengthen the idea that cytochrome *b₂* core and cytochrome *b₅* may have diverged from a common ancestral protein. Important questions to be answered are how has the counterpart of the cytochrome *b₅* hydrophobic tail evolved in cytochrome *b₂*, and whether the flavin-binding region in the yeast enzyme is homologous to cytochrome *b₅* reductase (see Fig. 1).

BERNARD GUIARD
FLORENCE LEDERER
C. JACO*

Centre de Génétique Moléculaire, CNRS,
91190 Gif-sur-Yvette, France

Received March 24; accepted April 15, 1975.

*Present address: MRC Laboratory of Molecular Biology, Hills Road, Cambridge, UK.

- ¹ Jacq, C., and Lederer, F., *Eur. J. Biochem.*, **41**, 311-320 (1974) and refs therein.
² Appleby, C. A., and Morton, R. K., *Nature*, **173**, 749-753 (1954).
³ Labeyrie, F., Di Franco, A., Iwatsubo, M., and Baudras, A., *Biochemistry*, **6**, 1791-1797 (1967).

- 4 Labeyrie, F., Groudinsky, O., Jacquot-Armand, Y., and Naslin, L., *Biochim. biophys. Acta*, **128**, 492-503 (1966).
- 5 Watari, H., Groudinsky, O., and Labeyrie, F., *Biochim. biophys. Acta*, **131**, 592-594 (1967).
- 6 Keller, R. M., Groudinsky, O., and Wüthrich, K., *Biochim. biophys. Acta*, **328**, 233-238 (1973).
- 7 Groudinsky, O., *Eur. J. Biochem.*, **18**, 480-484 (1971).
- 8 Guiard, B., Groudinsky, O., and Lederer, F., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 2539-2543 (1974).
- 9 Mevel-Ninio, M., *Eur. J. Biochem.*, **25**, 254-261 (1972).
- 10 Guiard, B., Groudinsky, O., and Lederer, F., *Eur. J. Biochem.*, **34**, 241-247 (1973).
- 11 Naslin, L., Spyridakis, A., and Labeyrie, F., *Eur. J. Biochem.*, **34**, 268-283 (1973).
- 12 Ozols, J., *Biochemistry*, **13**, 426-434 (1974).
- 13 Ito, A., and Sato, R., *J. biol. Chem.*, **243**, 4922-4923 (1968).
- 14 Spatz, L., and Strittmatter, P., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 1042-1046 (1971).
- 15 Shimakato, T., Mihara, K., and Sato, R., *J. Biochem., Tokyo*, **72**, 1163-1174 (1972).
- 16 Strittmatter, P., Rogers, M. J., and Spatz, L., *J. biol. Chem.*, **247**, 7188-7194 (1972).
- 17 Mathews, F. S., Argos, P., and Levine, M., *Cold Spring Harb. Symp. quant. Biol.*, **35**, 387-395 (1971).
- 18 Lederer, F., and Simon, A. M., *Eur. J. Biochem.*, **20**, 469-474 (1971).
- 19 Ozols, J., *J. biol. Chem.*, **247**, 2242-2245 (1972).
- 20 *Atlas of Protein Sequence and Structure* (edit. by Dayhoff, M.O.), (National Biomedical Research Foundation, 1972).
- 21 Spatz, L., and Strittmatter, P., *J. biol. Chem.*, **248**, 793-799 (1973).
- 22 Mihara, K., and Sato, R., *J. Biochem.*, **71**, 725-735 (1972).

Molecular conservation of 74 amino acid sequence of ubiquitin between cattle and man

UBIQUITIN (originally 'ubiquitous immunopoietic polypeptide' or 'UBIP')¹ induces lymphocyte differentiation and was first found in bovine thymus during isolation of the thymic polypeptide hormone thymopoietin (originally 'thymin')² which induces T-cell differentiation³ and has secondary effects on neuromuscular transmission². Ubiquitin, which has a molecular weight of 8,451, is interesting for several reasons. First, it induces differentiation of T cells and, unlike thymopoietin, B cells¹. Second, both properties are inhibited by the β -adrenergic blocking agent propranolol, and so the capacity of ubiquitin to induce lymphocyte differentiation seems to depend on reaction with a β -adrenergic receptor. Third, ubiquitin activates adenyl cyclase in lymphocyte precursors and in other tissues, again through a β -adrenergic receptor (M. Bitensky, and G. G., unpublished). Finally, ubiquitin, as the name implies, is found not only in thymus but in all other animal cells and in yeasts, bacteria and higher plants¹. Although its physiological function remains unknown, ubiquitin must be vital to the living cell to have

been conserved over such a long evolutionary time span. We have reported the complete primary structure of bovine ubiquitin⁴—a single polypeptide chain of 74 amino acids—and now report that human ubiquitin has an identical sequence.

Human ubiquitin was isolated from thymus obtained from cases of sudden death in young people, the purification procedures being as described for bovine ubiquitin¹ except that the thymus was initially homogenised at a lower concentration (5% wet weight per volume). The product was pure by polyacrylamide gel disc electrophoresis¹ and end-group analysis.

Residues 1–56 were identified by automated Edman degradations of 6 mg using a Beckman model 890 sequencer as described previously⁴. The average repetitive yield was 92%. Human ubiquitin was maleated to block the lysine residues and cleaved with trypsin at the arginine residues as for bovine ubiquitin¹. The four resulting peptides were resolved by molecular sieve chromatography on BioGel P-6 in a pattern identical to that obtained with bovine ubiquitin¹; they were designated MT-1, MT-2, MT-3 and MT-4 in order of decreasing size. The amino acid content and N-terminal analyses of intact human ubiquitin and the tryptic peptides of maleated human ubiquitin (Table 1) are identical to those of bovine ubiquitin⁴. Peptide MT-1, consisting of 42 amino acids and with a blocked N-terminal residue (maleated) corresponds to the N-terminal portion of ubiquitin. Peptides MT-2, MT-3 and MT-4 were fully sequenced by manual Edman degradations with positive identifications at each position. The automated sequence determination of human ubiquitin provided the sequence corresponding to MT-1 and MT-3 and the overlap provided an order of MT-1, MT-3 and MT-2. MT-4 was placed at the C-terminus by exclusion and by homology with bovine ubiquitin. The complete amino acid sequence of human ubiquitin is summarised in Fig. 1.

The evolutionary rate of change for amino acid sequences differs greatly among various proteins⁵. The slowest-changing sequence yet recorded is histone IV with only two substitutions in 102 residues between cattle and pea⁶. Cytochrome c, the change of sequence of which is considered relatively slow, has 10 substitutions in 112 residues between cattle and man⁷. In comparison, the identity of bovine and human ubiquitin sequences is remarkable. Since many substitutions entail only minimal structural change one can only wonder

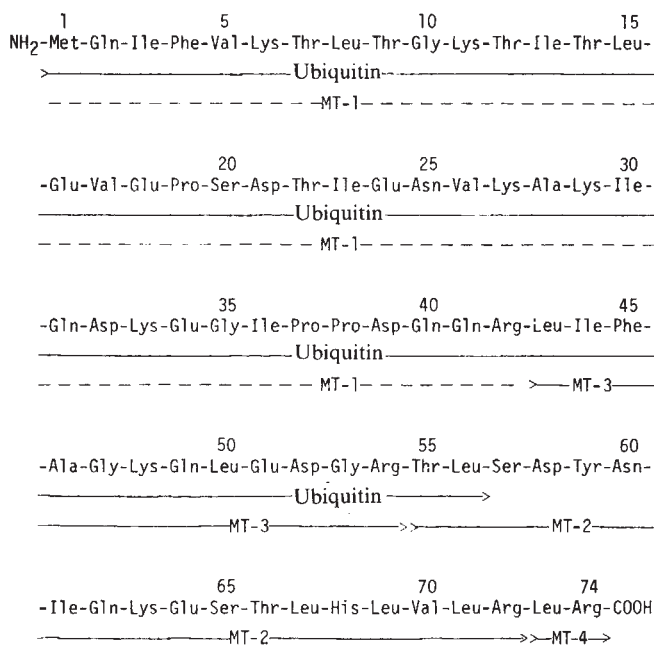


Fig. 1 Complete amino acid sequence of human ubiquitin. Arrows to the right indicate amino acids identified directly from Edman degradations with each arrow bearing a notation indicating the peptide studied (see text). The extent of peptide MT-1 is indicated by a broken line.

what structural stringencies imposed on ubiquitin within the living organism are so vital as to require full conservation of a 74 amino acid sequence within these two orders of mammals.

This work was supported by funds from the US Public Health Service and the Irvington House Institute. Ronald King, Miriam Miller and Philip Dee provided assistance.

DAVID H. SCHLESINGER

Endocrine Unit, Department of Medicine,
Massachusetts General Hospital,
Boston, Massachusetts 02114

GIDEON GOLDSTEIN

Memorial Sloan-Kettering Cancer Center,
New York, New York 10021

Received March 5, 1975.

- Goldstein, G., et al., *Proc. natn. Acad. Sci. U.S.A.*, **72**, 11 (1975).
- Goldstein, G., *Nature*, **247**, 11 (1974).
- Basch, R. S., and Goldstein, G., *Proc. natn. Acad. Sci. U.S.A.*, **71**, 1474 (1974).
- Schlesinger, D. H., Goldstein, G., and Niall, H. D., *Biochemistry* (in the press).
- Dayhoff, M. O. (ed.), *Atlas of protein sequence and structure* (National Biomedical Research Foundation, Washington, DC, 1972).
- Delange, R. J., Fambrough, D. M., Smith, E. K., and Bonner, J., *J. biol. Chem.*, **244**, 5669 (1969).

Table 1 Amino acid composition and N-terminal analysis of human ubiquitin and the tryptic peptides of maleated human ubiquitin

	Ubiquitin	MT-1	MT-2	MT-3	MT-4
Asp	6.9(7)	4.3(4)	2.0(2)	1.2(1)	0.1(0)
Thr	7.2(7)	4.6(5)	1.9(2)	0.2(0)	0.1(0)
Ser	2.9(3)	0.7(1)	1.7(2)	0.0(0)	0.0(0)
Glu	12.4(12)	7.6(8)	2.4(2)	2.2(2)	0.1(0)
Pro	3.4(3)	3.1(3)	0.2(0)	0.0(0)	0.0(0)
Gly	4.3(4)	2.2(2)	0.2(0)	2.3(2)	0.2(0)
Ala	2.2(2)	1.3(1)	0.1(0)	1.2(1)	0.1(0)
† Cys	0.0(0)	0.0(0)	0.0(0)	0.0(0)	0.0(0)
Val	4.2(4)	3.4(3)	1.0(1)	0.1(0)	0.0(0)
Met	0.9(1)	0.8(1)	0.1(0)	0.1(0)	0.0(0)
Ile	6.9(7)	4.8(5)	1.0(1)	1.1(1)	0.1(0)
Leu	9.1(9)	2.4(2)	4.0(4)	2.3(2)	1.0(1)
Tyr	0.9(1)	0.2(0)	0.9(1)	0.1(0)	0.0(0)
Phe	1.9(2)	1.0(1)	0.1(0)	1.0(1)	0.1(0)
Trp	0.0(0)	0.0(0)	0.0(0)	0.0(0)	0.0(0)
Lys	7.2(7)	5.1(5)	1.1(1)	0.1(1)	0.1(0)
His	1.0(1)	0.2(0)	0.9(1)	0.0(0)	0.0(0)
Arg	4.2(4)	1.3(1)	1.0(1)	1.1(1)	0.9(1)
N-terminus	Met	—	Thr	Leu	Leu
Total	74	42	18	12	2

The composition of each peptide is given as the molar ratios of the amino acids; figures in parentheses are the assumed numbers of residues. These findings are identical to those for bovine ubiquitin⁴.

Hybrid troponin reconstituted from vertebrate and arthropod subunits

TROPONIN, a regulatory protein modifying muscular contraction is found in all the chordate striated muscles as well as in many of the invertebrate muscles which have been studied¹⁻³. The complex of troponin and tropomyosin is bound to the actin containing thin filaments, and both components are required for thin filament-linked regulation^{1-3,10}. In the absence of calcium the troponin-tropomyosin complex blocks the myosin combining sites, inhibiting ATPase activity and therefore contraction. When present in micromolar amounts, calcium binds to troponin and releases the inhibition. Vertebrate troponin

consists of three subunits: TN-I, TN-C, and TN-T (ref. 4). TN-I by itself (molecular weight 24,000) inhibits the actin-myosin interaction irrespective of calcium ion concentration. TN-C (molecular weight 18,000) binds calcium ions and this releases the TN-I imposed inhibition. TN-T (molecular weight 37,000) attaches the subunit complex to tropomyosin. The troponin found on arthropod thin filaments is also composed of subunits^{3,5,6}. Components with chain weights of 29,000 and 18,000 have features analogous to those of vertebrate TN-I and TN-C respectively⁶. A third component is also found in arthropod troponin preparations (chain weight 55,000–59,000), but its function is not yet fully understood^{5,6}. For convenience, this chain will be referred to here as TN-T. In spite of the overall similarity of vertebrate and arthropod troponin, invertebrate troponin differs from vertebrate troponin in calcium binding properties, and binds one-half to one-quarter of the calcium in conditions where the thin filament is maximally switched-on^{3,6}. Moreover, the thin filament-based regulation in invertebrates is usually coupled with a myosin-linked regulatory system which is apparently not present in chordate striated muscle^{3,10}.

It is thought that there was a divergence early in the evolution of the animal phyla leading separately to the chordates and to the segmented invertebrates. Since arthropods and vertebrates have separate lineages, the troponin in these phyla may represent examples of convergent evolution, or may have evolved from a common ancestor. One approach for distinguishing between these alternatives, is to determine whether or not interphyletic hybrids can be made and are functional. In this study I show that a functional troponin hybrid can be made from rabbit TN-I and TN-T and *Limulus* TN-C.

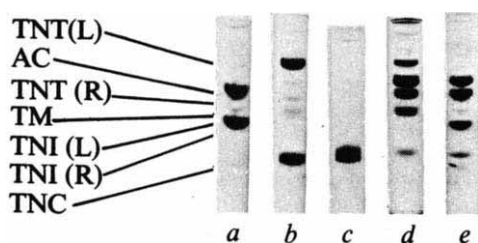


Fig. 1 10% SDS polyacrylamide gels⁹ using Coomassie Brilliant Blue stain. *a*, Rabbit TN-I-T, 10 μ g. *b*, *Limulus* TN-C-T, 11 μ g. *c*, *Limulus* TN-C, 15 μ g. *d*, *Limulus* thin filaments, 30 μ g. *e*, Reconstituted thin filament complex of rabbit actin, chicken tropomyosin, rabbit TN-I-T, and *Limulus* TN-C (proteins mixed in a 1.0:0.5:0.5:1.3 weight ratio in 40 mM NaCl, 1 mM MgCl and sedimented at 160,000g for 2 h, and the pellet used for gel electrophoresis). This gel shows approximately 10 μ g actin. (R) and (L) denote rabbit and *Limulus*, and AC and TM represent actin and tropomyosin. All proteins can be resolved into separate bands except TM and TN-T(R) on gel *e*.

Rabbit actin, myosin, and chicken tropomyosin were prepared as previously described^{6,7}. Rabbit and *Limulus* troponins were prepared according to Regenstien and Szent-Györgyi⁶. A fraction containing rabbit TN-I and TN-T (TN-I-T) (Fig. 1a) was prepared essentially by the method of Greaser and Gergely⁴. TN-I and TN-T were eluted together with the void volume from DEAE-Sephadex A-50 equilibrated with 100 mM NaCl, 2 mM EDTA, 6 M urea, 0.03% β -mercaptoethanol, 50 mM Tris pH 8.0. A preparation containing primarily *Limulus* TN-C and TN-T (TN-C-T) (Fig. 1b) could be prepared either by the method of Regenstien and Szent-Györgyi⁶ or by a second method in which an equal weight of DEAE-Sephadex A-50 is added to the *Limulus* troponin and the mixture dialysed against the buffer described above. The Sephadex is sedimented and the TN-I remains in the supernatant. The TN-C-T can be

removed from the Sephadex by sedimentation in the same solution containing 0.6 M NaCl. Conventional methods used to prepare vertebrate TN-C, when applied to arthropods, yield a TN-C contaminated with variable amounts of TN-T. To prepare a purer TN-C (Fig. 1c) an equal weight of SP-Sephadex C-50 is added to the troponin and the mixture dialysed against 90 mM NaCl, 6 M urea, 0.03% β -mercaptoethanol, 10 mM sodium acetate pH 5.0. The Sephadex is sedimented and the TN-C is in the supernatant. This method was also used to prepare rabbit TN-C except that the pH of the buffer was adjusted to 6.0. The fractionated *Limulus* subunits and the rabbit TN-C were then dialysed against 40 mM NaCl, 1 mM MgCl₂, 5 mM phosphate buffer pH 7.0, and small amounts of precipitate were removed by centrifugation and discarded. The rabbit TN-I-T precipitates in these conditions and was redissolved in 0.6 M NaCl.

Table 1 ATPase activities of actomyosin preparations

Troponin subunits added	Mg-ATPase		Calcium sensitivity 100-(ATPase EGTA)/100 ATPase Ca
	EGTA	Calcium	
(μmol ATP split per min per mg myosin)			
<i>a</i> , None	0.5	0.5	0
<i>b</i> , Rabbit TN-I-T	0.04	0.04	0
<i>c</i> , Rabbit TN-C*	0.47	0.47	0
<i>d</i> , Rabbit TN-I-T Rabbit TN-C*	0.14	0.57	75
<i>e</i> , <i>Limulus</i> TN-C-T†	0.33	0.30	0
<i>f</i> , <i>Limulus</i> TN-C‡	0.39	0.35	0
<i>g</i> , Rabbit TN-I-T <i>Limulus</i> TN-C-T†	0.12	0.37	68
<i>h</i> , Rabbit TN-I-T <i>Limulus</i> TN-C‡	0.19	0.39	52

In each ATPase assay 0.26 mg rabbit F-actin was mixed with 0.14 mg chicken tropomyosin in 40 mM NaCl, 1 mM MgCl₂, 5 mM phosphate buffer pH 7.0, brought to 0.4–0.6 M NaCl, and then mixed with 0.14 mg TN-I-T, or TN-C, TN-C-T or combinations of these at 0.4–0.6 M NaCl. Finally, 0.8 mg rabbit myosin was added. The mixture was then diluted in 10 ml 0.7 mM ATP, 1 mM MgCl₂, pH 7.5, 25 °C having a final NaCl concentration of 40 mM, and the ATPase assayed using the pH stat method as described previously^{7,8}. The calcium dependence was determined by comparing the ATPase rates in 0.1 mM EGTA and in 0.1 mM CaCl₂.

*0.08 mg

†0.4 mg

‡0.3–0.6 mg (variation in range maximally effective in different preparations).

It is well established that the addition of rabbit TN-I-T to tropomyosin-containing actomyosin results in a calcium independent inhibition of ATPase activity (Table 1b) and calcium sensitivity is restored when rabbit TN-C is also added (Table 1d) (compare ref. 4). In these studies it was found that a functional troponin is reconstituted by combining these fractions in 0.4–0.6 M NaCl and it was not necessary to mix the proteins in urea solution. The TN-I-T can be repeatedly frozen and thawed without losing inhibitory activity or its ability to complex functionally with TN-C.

Functional troponin hybrids can be made by mixing rabbit TN-I-T with *Limulus* TN-C-T or with *Limulus* TN-C, and the hybrids confer a calcium dependence on tropomyosin-containing rabbit actomyosin (Table 1g,h). Actin and tropomyosin combine with the latter hybrid troponin to form a complex which is free of *Limulus* TN-T and TN-I (Fig. 1e).

The amount of *Limulus* TN-C necessary for forming functional hybrids with rabbit TN-I-T is greater than the comparable rabbit TN-C requirement (Table 1). At reduced *Limulus* TN-C levels only a fraction of the TN-I-T imposed inhibition is relieved, but this activity is calcium sensitive. These results may indicate that the *Limulus* TN-C has a lower affinity for rabbit TN-I-T than rabbit TN-C, or that some of the *Limulus* TN-C may bind to actin-tropomyosin nonspecifically. It is also possible that the *Limulus* TN-C

preparation may be partly denatured; however, it should be noted that dialysis of whole troponin against 6 M urea at pH 5.0 does not diminish its activity, nor is the activity of *Limulus* TN-C altered by repeating its purification twice.

Since *Limulus* TN-C can substitute for rabbit TN-C and participate in forming a calcium sensitive rabbit actomyosin ATPase, it seems likely that the subunits of vertebrate and arthropod troponin function by the same mechanism and have therefore probably evolved from a common ancestor.

This work was supported by grants from the National Science Foundation and the US Public Health Service.

Note added in proof: A complementary experiment was performed in which chicken TN-C-T and lobster TN-I were mixed; a functional hybrid was formed which conferred calcium sensitivity on vertebrate actomyosin.

WILLIAM LEHMAN

Department of Physiology,
Boston University School of Medicine,
Boston, Massachusetts 02118

Received March 4; accepted April 2, 1975.

- ¹ Ebashi, S., and Endo, M., *Prog. Biophys. molec. Biol.*, **18**, 123-183 (1968).
- ² Murray, J., and Weber, A., *Physiol. Rev.*, **53**, 612-673 (1973).
- ³ Lehman, W., Kendrick-Jones, J., and Szent-Györgyi, A. G., *Cold Spring Harb. Symp. quant. Biol.*, **37**, 319-330 (1972).
- ⁴ Greaser, M. L., and Gergely, J., *J. biol. Chem.*, **246**, 4226-4233 (1971).
- ⁵ Bullard, B., Dabrowska, R., and Winkelman, L., *Biochem. J.*, **135**, 277-286 (1973).
- ⁶ Regenstein, J., and Szent-Györgyi, A. G., *Biochemistry*, **14**, 917-925 (1975).
- ⁷ Kendrick-Jones, J., Lehman, W., and Szent-Györgyi, A. G., *J. molec. Biol.*, **54**, 313-326 (1970).
- ⁸ Lehman, W., Bullard, B., and Hammond, K., *J. gen. Physiol.*, **63**, 553-563 (1974).
- ⁹ Weber, K., and Osborn, M., *J. biol. Chem.*, **244**, 4406-4412 (1969).
- ¹⁰ Lehman, W., and Szent-Györgyi, A. G., *J. gen. Physiol.* (in the press).

Self-assembly of lens crystallins *in vitro*

THE lens of vertebrate eye is very useful for the study of a number of fundamental biochemical processes at the molecular level. In this avascular tissue a continuous process of cellular differentiation takes place starting in the outer epithelial monolayer and ending in the lens centre, which consists of closely packed fibre cells. Strictly speaking, no cell death occurs in the lens, although differentiation from epithelium to fibre is accompanied by a gradual loss of cell organelles such as mitochondria, nuclei and ribosomes. Also, a very slow but defined breakdown of proteins starting from the C terminus^{1,2}, and deamidation of crystallins during ageing have been reported³.

We have shown previously that after treatment with 7 M urea the major structural lens protein α crystallin (molecular weight about 800,000) could be dissociated into subunits of molecular weight 20,000. After removal of urea the correct reassociation of subunits was achieved^{4,5}. Although the isolated β crystallin fraction could also be deaggregated, reassociation of this class of lens proteins failed, only insoluble aggregates being recovered. Eventually the complete mixture of all water-soluble lens proteins was also treated with urea and about 20 different monomeric polypeptides (20,000-30,000) were obtained. On dissociating the total mixture of 'native' crystallins, however, selective reassociation into α , β and γ crystallin could not be achieved. Since the observation that even enzymic activity (for example, ribonuclease⁶) could be restored after complete unfolding with urea, renaturation could be achieved by removal of the denaturing agent under well defined conditions⁶. Wetlaufer and Ristow⁷ stressed that the conformation of a protein depends on the kinetics as well as on the energetics of the folding process. On the other hand, the correct reassociation of groups of differing well defined protein assemblies in a complex mixture of highly hydrophobic components has not, to our knowledge, been reported previously. In a cell-free system derived from calf eye lens⁷, it seems that α , β and γ crystallins were synthesised and sorted out into proper assemblies. As, after translation, the individual proteins are presumably released from the ribosomes as monomeric polypeptides, it should be possible to detect conditions which promote the correct assembly of the subunits. The

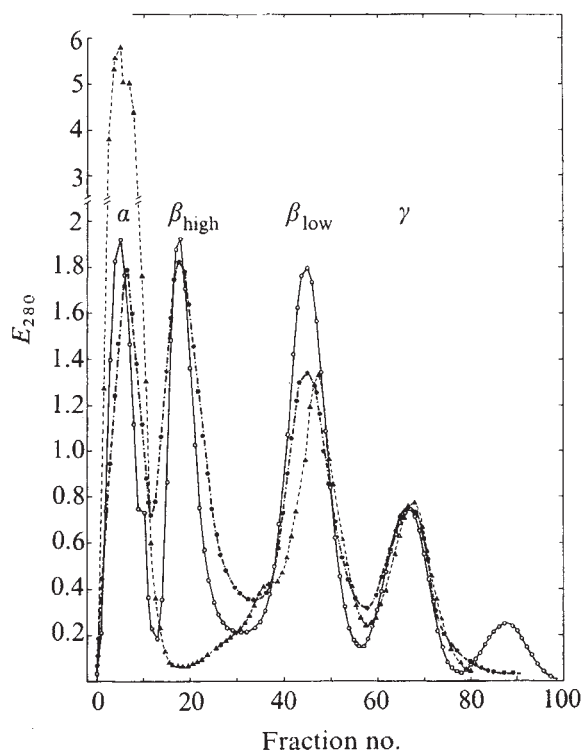


Fig. 1 Gel filtration patterns of native and renatured lens crystallins on Sephadex G-200. O, Native crystallins; ▲, Crystallins reassociated at a protein concentration of 200 mg ml⁻¹; *Crystallins reassociated at a protein concentration of 2 mg ml⁻¹. Column dimensions 100×4 cm. Equilibration and elution buffer 0.05 M Tris-HCl containing 0.051 M NaCl and 0.001 M EDTA (ref. 12) at pH 7.6. Elution was performed at room temperature for 40 h. Flow rate 18 ml h⁻¹. Preparation of total water-soluble lens extract has been described previously¹³.

present experiments show why we failed in 1962 and how 100% renaturation starting from a highly complex mixture of monomeric subunits can be achieved.

The water-soluble lens proteins can be separated by gel filtration on a Sephadex G-200 column. Four fractions are obtained, the first peak eluting with the void volume being α crystallin, followed by β_{high} , β_{low} and γ crystallin (average molecular weights 800,000 150,000, 50,000 and 20,000, respectively; Fig. 1). When the mixture of water-soluble lens proteins (200 mg ml⁻¹, optimal for separation by gel filtration in our experimental conditions) is dissociated with 7 M urea, dialysed against large volumes of deionised water, lyophilised, redissolved in 0.05 M Tris-HCl buffer (pH 7.6) containing 0.05 M NaCl and 0.007 M EDTA, and reapplied to a Sephadex G-200 column, another separation pattern, as obtained with native lens protein, is observed (Fig. 1). The β_{high} fraction has disappeared almost completely and β_{low} fraction has been significantly decreased. The peak fractions from the separations depicted in Fig. 1 were isolated and subjected to electrophoresis in polyacrylamide gels containing 7 M urea to obtain the subunit patterns shown in Fig. 2.

Reaggregation has clearly resulted from complexing of α and β components (Fig. 2b and 2d). γ Crystallin is not involved in complex formation (Fig. 2h). These results were substantiated by electron microscopy and immunochemical studies⁹. Of the various conditions for correct reassembly studied (such as pH, temperature, time dependence or dilution after removal of urea) only the protein concentration at the moment of urea removal determined proper or incorrect assembly. Thus, if a mixture of the naturally occurring crystallin polypeptides at any concentration between 200 and 20 mg ml⁻¹ was treated with 7 M urea the only prerequisite for correct recombination was dilution of the protein solution with 7 M urea to a final concentration of 2 mg ml⁻¹ before dialysis (Fig. 1). The gel

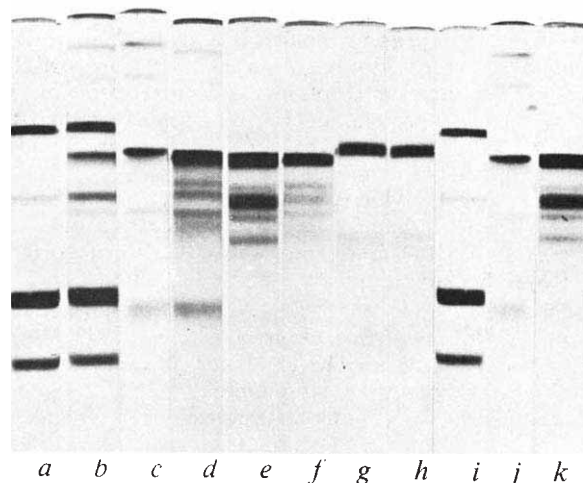


Fig. 2 Electrophoretic analysis of the separated water-soluble lens proteins on 7.5% polyacrylamide gels containing 7 M urea. *a*, Native α crystallin; *b*, $\alpha\beta$ hybrid; *c*, native β_{high} ; *d*, $\beta_{\text{high}}\beta_{\text{low}}$ hybrid; *e*, native β_{low} ; *f*, β_{low} reassociated; *g*, native γ ; *h*, γ reassociated (note that only the γ fraction is not involved in complex formation); *i*, correctly reassembled α crystallin fraction; *j*, correctly reassembled β_{high} crystallin fraction; *k*, correctly reassembled β_{low} crystallin fraction. *b*, *d*, *f*, and *h* show the electropherograms of α , β_{high} , β_{low} and γ crystallin reassociated at 200 mg ml⁻¹ protein and *i*, *j* and *k* at 2 mg ml⁻¹. γ Crystallin, which in all cases is unaltered, is not shown. Electrophoresis was performed as described elsewhere¹⁴.

filtration pattern shows that all crystallin components have formed. Gel electrophoresis proves that these renatured crystallins contain the correct polypeptides and that no hybrid formation took place. The actual concentration of the individual polypeptide chains therefore seems to provide a rather trivial but efficient regulatory mechanism for correct *in vitro* assembly of high polymeric proteins. Moreover, the results can also be interpreted by assuming that refolding of the monomeric polypeptides at low concentration is a first order reaction whereas the rate of interchain interaction is of a higher order. In this case proper interchain interaction of all crystallin polypeptides precedes the recombination process. Such a mechanism has recently been reported for renaturation of the hormones, bovine thyrotropin¹⁰ and ovine interstitial cell stimulating hormone¹¹.

Note that the selective recombination cannot be achieved after dissociation of the lens proteins into subunits by either acid or alkaline treatment. On neutralisation even at extremely high dilution only nonspecific reaggregation of α and β crystallin chains could be observed.

In vivo, another mechanism may be operative. If one assumes that proper folding of the peptide chains takes place while still on the ribosomes, correct assembly of the individual chains into the native polymer would occur. If, however, the folding of the individual chains occurs after release from the ribosomes the proper assembly may be regulated by the actual (low) concentration.

Our findings, although providing the tool for correct *in vitro* assembly, do not allow definite conclusions for the *in vivo* situation to be drawn.

HANS BLOEMENDAL
ANNEKE ZWEERS

Department of Biochemistry,
University of Nijmegen,
Geert Grooteplein Noord 21

HANS WALTERS

Department of Biophysical Chemistry,
University of Nijmegen,
Toernooiveld, Nijmegen, The Netherlands

Received February 20; accepted April 3, 1975.

- ¹ De Jong, W. W., Van Kleef, F. S. M., and Bloemendal, H., *Eur. J. Biochem.*, **48**, 271 (1974).
- ² Van Kleef, F. S. M., Nijzink-Maas, M. J. C. M., and Hoenders, H. J., *Eur. J. Biochem.*, **48**, 563 (1974).
- ³ Bloemendal, H., Berns, A. J. M., Van der Ouderaa, F., and De Jong, W., *Expl Eye Res.*, **14**, 80 (1972).
- ⁴ Bloemendal, H., Bont, W. S., Jongkind, J. F., and Wisse, J. H., *Nature*, **193**, 437 (1962).
- ⁵ Bloemendal, H., Bont, W. S., Jongkind, J. F., and Wisse, J. H., *Expl Eye Res.*, **1**, 300 (1962).
- ⁶ Anfinsen, C. B., and Haber, E., *J. biol. Chem.*, **236**, 1361 (1961).
- ⁷ Wetlaufer, D. B., and Ristow, S., *A. Rev. Biochem.*, **42**, 135 (1973).
- ⁸ Strous, G. J. A. M., Van Westreenen, H., Van der Logt, J., and Bloemendal, H., *Biochim. biophys. Acta*, **353**, 89 (1974).
- ⁹ Bloemendal, H., Zweers, A., Benedetti, E. L., and Walters, H., *Expl Eye Res.* (in the press).
- ¹⁰ Ingham, K. C., Aloj, S. M., and Edelhoch, H., *Archs Biochem. Biophys.*, **163**, 589 (1974).
- ¹¹ Bewley, T. A., Sairam, M. R., and Li, C. H., *Archs Biochem. Biophys.*, **163**, 625 (1974).
- ¹² Testa, M., Armand, G., and Balazs, E. A., *Expl Eye Res.*, **4**, 327 (1965).
- ¹³ Bloemendal, H., and Ten Cate, G., *Archs Biochem. Biophys.*, **84**, 512 (1959).
- ¹⁴ Bloemendal, H., in *Zone Electrophoresis in Blocks and Columns* (Elsevier Monographs: Chemistry Section 28 Amsterdam).

A highly α -helical structure protein in sarcoplasmic reticulum membranes

AMONG the various protein fractions isolated from sarcoplasmic reticulum (SR) membranes, MacLennan *et al.*¹ demonstrated the existence of a proteolipid of molecular weight about 12,000, that is, a protein which is soluble in 2:1 chloroform-methanol mixture^{2,3}. Recent spin-labelling (P. L. and M. D. Barratt, unpublished) and black-film experiments (P. L. and D. E. Graham, unpublished) have provided evidence that the proteolipid resides in the hydrocarbon interior of the phospholipid bilayer and reduces the alkyl-chain mobility. The apparent lack of any specific action in the Ca²⁺-transport process of SR suggests that the proteolipid merely provides structural support, possibly by stabilising the vectorial character of the transport system within the dynamic phospholipid matrix. Considering the widespread presence of proteolipids in biological membranes³, structural information about this type of intrinsic protein is of particular interest. With intrinsic membrane proteins the choice of methods for obtaining such direct information is restricted by the fact that in the native state the protein is associated with lipid molecules in aggregates of comparatively large dimensions. In this situation data on the secondary structure from measurement of the circular dichroism may be used to infer a model for the arrangement of the protein within the membrane.

Methods of preparing proteolipid from rabbit skeletal muscle, SR phospholipids and recombined systems were essentially as described previously^{1,2}. Circular dichroism spectra in the far ultraviolet range were obtained using proteolipid in methanol, and in aqueous dispersions of SR phospholipids and lysophosphatidylcholine (LPC). The micellar and vesicular character of the LPC and sonicated SR-phospholipid dispersions was confirmed by negative stain electron microscopy. The average Stoke's radius of the proteolipid-containing SR-phospholipid vesicles (by Sepharose 4-B column chromatography⁴) was 76 Å. Because of this small particle size no corrections^{5,6} for differential light scattering and absorption flattening were made, because at wavelengths higher than 200 nm such effects would be rather small and hardly affect conclusions drawn.

Comparison of the spectra (Fig. 1) with those of model polypeptides of known conformation⁷ reveals the typical features of a predominantly right-handed α -helical conformation in all three systems. Absorption flattening may be responsible for the relatively low positive maximum near 195 nm, although there seems to be no redshift caused by the particulate nature in the aqueous dispersions. Thus, an estimation of the α -helical proportion can be made from the ellipticities at 222 nm (ref. 8). The resulting values (Fig. 1, insert) are distinctly higher than those of most globular, water-soluble proteins⁹ in all three environments. The fact that the α -helical character is maintained in

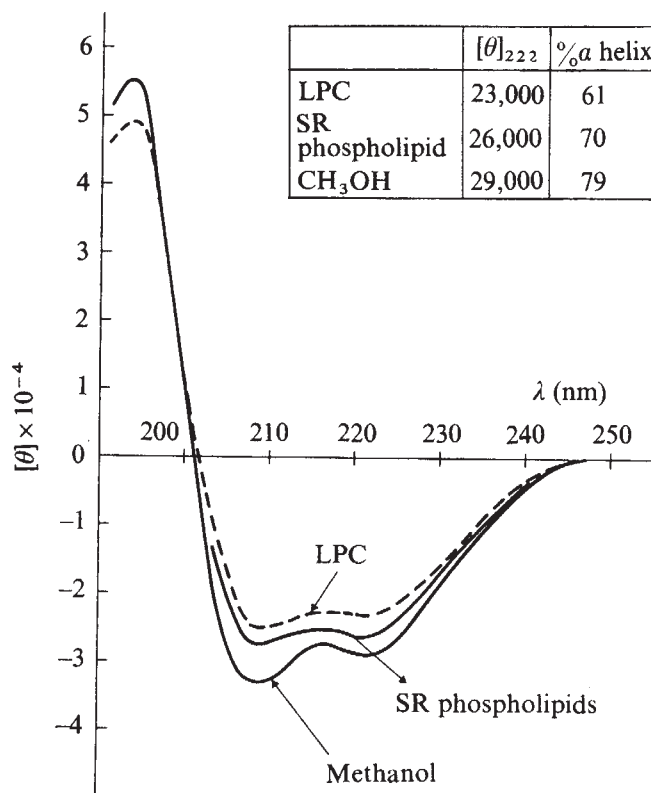


Fig. 1 Far ultraviolet circular dichroism spectra of SR proteolipid in methanol and in aqueous dispersions of membrane phospholipids and LPC (from egg yolk lecithin, Lipid Products, South Nutfield, UK), measured on a Cary 60 spectropolarimeter equipped with a Cary 6001 CD-accessory. Blanks measured with solutions of all components except the proteolipid were subtracted. The molar ellipticities $[\theta]$ in deg cm² dmol⁻¹ were calculated from $[\theta] = \text{mean residue weight} \times (\theta/10lc)$, where θ is the observed ellipticity, l is the optical path length in cm, c is the protein concentration in g cm⁻³ determined by the Lowry¹¹ method using bovine serum albumin as a standard, and the mean residue weight was taken as 119.3 (calculated from the amino acid composition¹). Weight ratios of protein-SR phospholipids, and protein-LPC were 1:50 and 1:20, respectively. Dispersions of SR phospholipid and proteolipid were sonicated under N₂ and ice-cooled for 20 min in volumes of 1 ml using a microsonprobe (MSE, England). The α -helical fraction was calculated from $[\theta]_{222}$ according to Glaser and Singer⁸.

methanol solution indicates that the conditions of proteolipid preparation do not interfere severely with the native conformation. Considering that the α -helical regions are essentially of cylindrical or rod-like shape, the relatively low α -helix fraction in the LPC-system can be rationalised on the grounds of the spherical micellar structure of the aggregates, which does not allow the formation of extended α -helical stretches within its restricted low-polarity core. No such hindrance prevails in the bilayer structure of the SR-phospholipid vesicles and, correspondingly, the α -helical proportion is higher there.

These results support the hypothesis that in the SR membrane the proteolipid is arranged predominantly in extended cylinders of α helices in the plane of the bilayer. The formation of a two-dimensional network of aggregated proteolipid molecules into which the phospholipid alkyl-chains are interdigitated would also explain the observed physical effects (P. L. and M. D. Barratt; and P. L. and D. E. Graham, unpublished) of a protein of relatively small molecular weight on a high number of phospholipid molecules.

It is likely that similar structures are present in other biological membranes containing proteolipid. The finding of Racker and Kandrach¹⁰ showing that a hydrophobic pro-

tein fraction from inner mitochondrial membranes, which by itself has no apparent biological activity, is essential to the functional reconstitution of an electron transport chain underlines the importance of such structural proteins. This type of intrinsic protein, of two-dimensional rather than corpuscular structure, may be considered in the discussion of current membrane models.

I thank Drs E. Morris and O. Korver from Unilever Research for their help. This work was carried out in the course of an EMBO fellowship, and was also supported by The Royal Society, London.

PETER LAGGNER

*Institut für Röntgenfeinstrukturforschung,
der Österreichischen Akademie der Wissenschaften,
und des Forschungszentrums Graz,
Steyrergasse 17, A 8010 Graz, Austria*

Received February 10; accepted March 27, 1975.

- MacLennan, D. M., Yip, C. C., Iles, G. M., and Seeman, P., *Cold Spring Harb. Symp. quant. Biol.*, **37**, 469-477 (1973).
- Folch, J., and Lees, M., *J. biol. Chem.*, **191**, 807-818 (1951).
- Folch-Pi, J., and Stoffyn, P. J., *Ann. N.Y. Acad. Sci.*, **195**, 86-107 (1972).
- Ackers, G. K., *J. biol. Chem.*, **242**, 3237-3238 (1967).
- Holzwarth, G., in *Membrane Molecular Biology* (edit. by Fox, C. F., and Keith, A. D.), 228-286 (Sinauer, Stamford, Connecticut, 1972).
- Masotti, L., Lenaz, G., Spisni, A., and Urry, D. W., *Biochim. biophys. Res. Commun.*, **56**, 892-897 (1974).
- Greenfield, N., and Fasman, G. D., *Biochemistry*, **8**, 4108-4116 (1969).
- Glaser, M., and Singer, S. J., *Biochemistry*, **10**, 1780-1787 (1971).
- Timasheff, S. N., et al., in *Conformation of Biopolymers* (edit. by Ramachandran, G. N.), 173 (Academic, New York, 1967).
- Racker, E., and Kandrach, A., *J. biol. Chem.*, **246**, 7069-7071 (1971).
- Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. biol. Chem.*, **193**, 265-275 (1951).

Errata

In the article "Chromosome imprinting and the mammalian X chromosome" by H. Sharat Chandra and S. W. Brown (*Nature*, **253**, 165; 1975), page 168, paragraph 2, line 1, for ref. 33 read ref. 32.

In the article "Energy spectrum of diffuse component of cosmic soft γ rays" by Y. Fukada, S. Hayakawa, I. Kasahara, F. Makino, Y. Tanaka and B. V. Sreekantan (*Nature*, **254**, 398; 1975) the ceiling heights given for the balloon in the fourth paragraph should read 430 and 680 Pa and not as printed.

Corrigenda

In the article "*Paracoccus denitrificans* and the evolutionary origin of the mitochondrion" by P. John and F. R. Whatley (*Nature*, **254**, 495; 1975) there is an error in the legend to Fig. 2. For Tris-acetate read Tris-phosphate.

In the article "Missing link between Milankovitch and climate" by G. J. Kukla (*Nature*, **253**, 600; 1975) the Q values in Table 1 are incorrect. The correct values are shown in the table reprinted below. These errors in no way affected the text, figures and conclusions that followed in the original article.

Table 1 Monthly means* of Q , T and S

	Q langley d ⁻¹	Globe			Northern Hemisphere			Southern Hemisphere		
		T °C	S 10 ⁶ km ²	S km ²	Q langley d ⁻¹	T °C	S 10 ⁶ km ²	Q langley d ⁻¹	T °C	S 10 ⁶ km ²
January	566	12.2	76	380	8.0	58.4	751	16.5	18	
April	547	14.1	59	644	14.2	41.2	451	14.1	18	
July	536	16.1	42	723	21.6	14.3	349	10.6	28	
October	548	14.3	57	480	16.2	22.8	616	12.4	34	
Mean	550	14.2	60	556	15.0	34.8	543	13.4	25	

* Q , After Berland¹⁰; T , long term means after Shutz and Gates¹¹; satellite observed mean S areas covered for at least five consecutive days in the Northern Hemisphere during 1967-73 interval and in the Southern Hemisphere in 1968 (ref. 14)

matters arising

No evidence for heritability of social attitudes

EAVES and Eysenck¹ have concluded that six types of personality orientations, including both radicalism and neuroticism, have substantial genetic components. These conclusions, based on simple models, are presented with some qualifications but not without conviction and certainty. The reader is informed that: "a model invoking only environmental factors fails to provide an adequate description of the variation and covariation of the six traits"; that, although a larger study would be desirable, "we can, however, be reasonably confident that some kind of genetic mechanism has to be involved to account for the observations so far"; and that the correct interpretation of a table presenting exact heritability estimates for each trait, is that "about half the variation between individuals for the six traits has a genetic basis".

None of these conclusions is justified by the data. Eaves and Eysenck's two-parameter environmental model tests only whether dizygotic twins are as similar in their personality scores as monozygotic twins. The high χ^2 for this model indicates that the monozygotic twins are more similar than dizygotic twins. Eaves and Eysenck simply assume that this extra similarity is genetic. In a paper² on the heritability of IQ performance we showed that comparisons between monozygotic and dizygotic twins can only set upper limits to the heritability of traits because of the greater similarity of treatment of monozygotic twins. We estimated the magnitude of this treatment effect and showed it to be comparable with the presumed genetic effect.

Eaves and Eysenck make the same error in the present study. They have assumed that monozygotic twins, who look alike and are regularly confused for each other, receive the same treatment as dizygotic twins who are rarely confused³ and they assume that the rejection of a primitive two-parameter environmental model means that no environmental model can be fitted to the data. Such procedures are inadequate. Comparisons between dizygotic twins of the same sex and dizygotic twins of the

opposite sexes, for example, should be reported to see if the differences are comparable with monozygotic-dizygotic differences. If so, this would indicate that environmental effects could account for greater monozygotic similarity and indicate an absence of genetic effects. Even better would be to compare dizygotic twins who look alike³ with monozygotic twins, to control for physical similarity.

The models used in this study and in IQ studies⁴ have come to substitute for sensible experimental controls and for adequate tests of competing hypotheses. The conclusions are unwarranted and misleading. They reflect only the assumptions of the authors and assume the very results they are trying to prove.

MICHAEL SCHWARTZ

*Department of Sociology,
State University of New York,
Stony Brook, New York 11790*

JOSEPH SCHWARTZ

*Division of Pure and Applied Sciences,
City University of New York,
Richmond College,
Staten Island, New York 10301*

¹ Eaves, L. J., and Eysenck, H. J., *Nature*, **249**, 288 (1974).

² Schwartz, M., and Schwartz, J., *Nature*, **248**, 84 (1974).

³ Cohen, D. J., Dibble, E., Grawe, J. M., and Pollin, W., *Archs gen. Psychiat.*, **29**, 465 (1973).

⁴ Jensen, A. R., *Proc. natn. Acad. Sci. U.S.A.*, **58**, 149 (1967).

Specificity in initiation of mRNA translation

LODISH¹ proposes a model for the regulation of mRNA translation in which changes in specificity may actually be provoked by a nonspecific change in a kinetic parameter of protein synthesis. I had developed a similar idea earlier^{2,3} in the discussion of ribosomal discrimination at the elongation level⁴ whereas Lodish discusses the initiation step. The mathematics of Lodish's paper seem to be inaccurate, in addition to the acknowledged simplifications.

Equation (1) applies to infinite chains only. Equation (3) cannot be correct; this can be checked by solving the steady-state equations in a simple case (for example, a messenger three codons in length where $L=2$). Combining these two leads to equation (5), which predicts negative rates of protein synthesis when initiatable ribosomes are in large excess ($K_e/K_1 R^* < 1$). Lodish does not claim that his treatment is valid for such extreme cases. The presence of the approximations casts doubt on the claim that the treatment takes into account the effect of hindrance to translocation of a ribosome as a result of the presence of an adjacent ribosome. When this effect is negligible, the correct rate equation can be established very simply. Consider an initial situation in which a ribosome has just moved from the L th to the $(L+1)$ th codon. The mRNA becomes initiatable. The average time which elapses before there is a collision with a ribosome is, by definition of the kinetic parameters of Lodish's model, $1/R^*K_1$. Completion of initiation then takes a certain time ϵ , and the L successive translocations take, by definition, a time L/K_e . The mRNA is now initiatable again and a new cycle begins. Thus, the average time (that is the reciprocal of the turn-over number) it takes for a mRNA molecule to produce a protein is:

$$t = 1/R^*K_1 + \epsilon + L/K_e \quad (1)$$

When ϵ is negligible, equation (1) can be written as:

$$QL/mK_e = L/tK_e = K_1/(K_1 + K_e/R^*L) \quad (2)$$

This kinetic treatment of specificity in

Matters arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

initiation of translation is isomorphous with my treatment of elongation (3) in which I had:

$$\rho = \theta / (\theta + \tau) \quad (3)$$

where θ (like K_1) characterises the strength of the association and τ (analogous to the R^* term) is the parameter that changes nonspecifically. A more sophisticated model has been considered recently⁵.

Substituting $(L-1)$ with L in the denominator of the right-hand expression of Lodish's equation (5) one gets my equation (2).

JACQUES NINIO

Laboratoire de Biochimie du
Développement,
Institut de Biologie Moléculaire du CNRS,
Université Paris VII,
2 Place Jussieu, 75005 Paris, France

¹ Lodish, H. F., *Nature*, **251**, 385-388 (1974).

² Ninio, J., *Progr. Nucleic Acid Res. Mol. Biol.*, **13**, 301-337 (1973).

³ Ninio, J., *J. molec. Biol.*, **84**, 297-313 (1974).

⁴ Gorini, L., *Nature new Biol.*, **234**, 261-264 (1971).

⁵ Ninio, J. in *L'évolution des Macromolécules Biologiques* (edit. by Sadron, C.) (Editions du CNRS, Paris, in the press).

DR LODISH REPLIES—Ninio's¹ equation (1) is incorrect on several grounds. First, it yields not the average time for an mRNA to produce a complete protein, but rather the average time it takes an mRNA to produce a polypeptide L amino acids long (L being the number of codons covered by a single ribosome). Secondly, it neglects, by his own admission, the case in which progress of one ribosome is hindered by the presence of another distal to it along the mRNA. As is clear from Fig. 1 of my paper², this parameter is considerable for usual densities of ribosomes along an mRNA such as occur in (uninhibited) reticulocytes. The fact that Ninio can transform his equation into one, his equation (3), which resembles my equation (5), does not prove that his equation is correct.

I did point out in my paper the several simplifying assumptions that were made in my derivations. In particular, I pointed out that the equations were valid only for cases in which the overall rate of chain initiation is the same or less than that which obtains in a normal cell. Thus, Ninio's comments about cases in which initiatable ribosomes are in large excess ($K_1 R^*/K_c > 1$) are irrelevant.

All my assumptions, although valid for reticulocytes, might not be for other types of cells. To remove these assumptions necessitates a much more complex statistical analysis of ribosome movement. These are in progress in my laboratory. But I am afraid Ninio's equations are incorrect and do not really help matters.

Cambridge, Massachusetts

¹ Ninio, J., *Nature*, **255**, 429-430 (1975).

² Lodish, H. F., *Nature*, **251**, 385-388 (1974).

Normal redshifts in Markaryan galaxies

TEERIKORPI claims to have found evidence for anomalous redshifts in Markaryan galaxies. His method was to take a sample consisting of the 394 measured redshifts of galaxies in Markaryan's first five lists. He then divided the redshifts into bins of $\Delta z = 0.001$, and, according to him, a peak in the resulting distribution at $z = 0.015-0.016$ is too large to be a chance effect.

Teerikorpi estimates the significance of this peak by calculating the probability that 30 or more redshifts would occur in an interval of $\Delta z = 0.002$, assuming that the distribution of redshifts between $z = 0.003$ and $z = 0.030$ is rectangular, and assuming that Poisson statistics hold. Teerikorpi estimates this probability as 0.05. This is incorrect. There are 269 galaxies in 27 bins of $\Delta z = 0.001$, or approximately 20 galaxies for each bin of $\Delta z = 0.002$. The probability, with these assumptions concerning the distribution, is 0.02 that 30 galaxies will be found in a given interval; however, there are 26 such bins, making the true probability 0.44 that this is just a chance distribution. Things are no better if one considers intervals of $\Delta z = 0.001$. The probability of 17 or more in a given bin is 0.03; for 27 bins it is 0.52. Including the peak at $z = 0.006$, which Teerikorpi does not mention as significant, the probability that the observed distribution results by chance is 0.28.

Teerikorpi argues that 0.015 is an integral fraction of the peaks at $z = 0.03$ and $z = 0.06$ found by Burbidge² and by Burbidge and O'Dell^{3,4}. No peak is found at 0.030 in Teerikorpi's sample, although it extends past that point, and no peak at 0.015 was found in the sample of galaxy redshifts used by Burbidge and O'Dell³ even though more than 40% of their sample were Markaryan galaxies.

The assumptions about the gross distribution of redshifts are poor. It is not rectangular, but is a curve peaked near $z = 0.020$. This is exactly as expected with the observed counting statistics as a function of limiting magnitude if one assumes that the distribution of Markaryan galaxies in space and brightness is similar to normal galaxies. Also, as pointed out by Huchra and Sargent⁵, Markaryan's survey does not yet cover a large enough area of the sky to remove adequately the small scale effects of clustering. Both of these effects will tend to decrease the significance of any clumps or peaks in the redshift distribution. Finally, because of the small number of samples (27 bins), and the interdependence of the samples, (a peak in one bin will cause a dip in others, with a fixed number of galaxies), it is not clear that Poisson statistics should be used. If a distribution with a larger half width is used, then the

significance of the peaks is decreased still further.

In the light of these arguments, I find nothing to support the claim for anomalous redshifts or non-cosmological components for Markaryan galaxies.

This work was supported in part by the NSF.

JOHN HUCHRA

Hale Observatories, CalTech,
Carnegie Institute of Washington,
Pasadena, California 91101

¹ Teerikorpi, P., *Nature*, **252**, 110-111 (1974).

² Burbidge, G. R., *Astrophys. J. Lett.*, **154**, L41-L48 (1968).

³ Burbidge, G. R., and O'Dell, S. L., *Astrophys. J.*, **178**, 583-605 (1972).

⁴ Burbidge, G. R., and O'Dell, S. L., *Astrophys. J. Lett.*, **186**, L59-L62 (1973).

⁵ Huchra, J., and Sargent, W. L. W., *Astrophys. J.*, **186**, 433-443 (1973).

DR TEERIKORPI REPLIES—My incorrect value resulted from an unfortunate elementary error in my calculations¹, and went unnoticed till now. I am grateful to Huchra for drawing my attention to it.

The low statistical significance of the peak does not, however, invalidate altogether my other arguments, which were the main reasons for suggesting anomalous redshifts. The peak as such was only marginally significant.

It is still interesting that this peak, which at the first sight is a natural candidate for a possible indicator of anomalous redshifts, is consistent with simple ideas concerning intrinsic redshifts: they tend to occur in compact objects, they should be more easily detected in the direction of near, dense galaxy populations, and they possibly prefer certain values connected with simple relationships. One cannot compare the results of Burbidge and O'Dell^{2,3} with my analysis. I did not divide the objects according to their radio or emission-line properties, only according to the ultraviolet classification of Markaryan. My sample also contained at least four times as many Markaryan objects as theirs did. Their first analysis of emission-line objects did not include any Markaryan galaxies in the relevant interval: 0.01-0.02.

It is harder to detect relatively small anomalous redshifts than large ones (such as in QSOs, if real) because in addition to the smearing factors I mentioned (random motions, cosmological recession) there are in this case many normal Dopplerian redshifts as 'noise' in redshift distributions. So in order to find out those properties which indicate intrinsic redshifts in objects one must work with a lower statistical significance level and use additional consistency tests. Otherwise possible anomalous redshift 'signals' may well go unnoticed.

Tahtitorninmaki,
SF-00130 Helsinki 13,
Finland

¹ Teerikorpi, P., *Nature*, **252**, 110-111 (1974).

² Burbidge, G. R., and O'Dell, S. L., *Astrophys. J.*, **178**, 583-605 (1971).

³ Burbidge, G. R., and O'Dell, S. L., *Astrophys. J. Lett.*, **186**, L59-L62 (1973).

reviews

THE bible on cholinesterase has for more than 10 years been the voluminous *Handbuch des Experimentellen Pharmakologie*, edited by G. B. Koelle in 1963. Dr Silver's book is very different in many ways, mainly because it is the work of a single author. Although enzymological aspects of cholinesterase fall within the scope of the book along with more physiological points, the different chapters are all clearly oriented towards the biology of cholinesterases. The title of the book therefore describes its contents quite aptly.

Following Lewis Carroll's advice to Alice in Wonderland ("Begin at the beginning, and go on till you come to the end: then stop") which she uses as an introductory quotation, Dr Silver, after a few paragraphs on the history of cholinesterase research describes the enzymological and biochemical properties of the enzyme. This chapter is written in a way that will be easily comprehensible to students or scientists who are not familiar with enzymology. The meaning of the catalytic parameters is explained, and chemical terms are defined in a glossary at the end.

After a clear description of neuronal morphology, the subcellular localisation of acetylcholinesterase is covered, as well as its transport along the axon, and the location of its biosynthesis. Cholinesterases in invertebrates are discussed and an abundance of data on histological distribution of cholinesterase in vertebrates is then presented in a series of detailed tables (120 pages).

Three chapters are devoted to a discussion of the biological role of

Cholinesterase end to end

J. Massoulié

The Biology of Cholinesterases. Frontiers of Biology, vol. 36.) By Ann Silver. Pp. xiv+596. (North-Holland: Amsterdam and Oxford; American Elsevier: New York, 1974.) Dfl.145; \$55.95.

cholinesterase. Nachmansohn's theory of a universal cholinergic conduction mechanism is discussed. Dr Silver then considers the question: what is the function of acetylcholinesterase in erythrocytes, in placenta, and in nervous tissue outside cholinergic synapses? She studies the factors affecting its activity: the level of acetylcholinesterase varies with the physiological condition of the animals; it is under endocrine influence, and can be altered by drugs, lesions or other experimental interference; evidence points to a possible control of acetylcholinesterase by acetylcholine at motor endplates.

The last two chapters are devoted to pseudocholinesterases, and anticholinesterases which, quite logically, have been placed at the end.

I have to mention particularly the chapter on methods, which gives a precise and very detailed description of biochemical assays and histochemical localisation procedures for cholinesterases. Variants of these methods are carefully compared, and this part of the book should be very useful.

Research on acetylcholinesterase has been progressing very actively in the last few years, and it was inevitable that some of the more recent acquisitions could not be incorporated: recent data, for example, on the molecular structure of electric eel and torpedo acetylcholinesterase, or on the relationship of a definite molecular form with neuromuscular endplates in the rat diaphragm could not be incorporated although they would have been quite relevant. Neither have the new methods of acetylcholinesterase purification based on specific ligand chromatography been described. But I think that the book has succeeded in its aim to provide for the physiologist an introduction to the biochemistry of cholinesterase and for the biochemist an introduction to the relevant physiological problems.

Dr Silver's book, although not perhaps as exhaustive as Koelle's treatise, will act as an extraordinary source of information: it includes more than 1,800 references. Much of the data are presented in tables allowing that the text remains free for discussions, and rendering it very readable. It must have been a difficult task to organise heterogeneous data in this form, but it provides an easy method of retrieving information, and allows useful comparisons. A subject index is provided, but an author index would also have been useful.

Although the book is not meant to be read from beginning to end at one time, it is written in a simple and pleasant way, and is a valuable acquisition for any scientist concerned with cholinesterase and cholinergic transmission.

THIS is a timely book, to be welcomed by workers and referees of papers in a field of widely dispersed literature. About half of it describes the constitution of natural and synthetic compounds which form lipid-soluble complexes with alkali and alkaline earth metal cations and stresses the vital importance of the three-dimensional structures in conferring the characteristic properties. Short chapters are devoted to methods of studying complex formation, with tables of results, and to possible uses of the phenomenon in chemistry which show how well Pedersen in 1967 signposted these aspects.

It is a genuine book, not a collection of papers, and has cohesion and per-

Complexones

Membrane-Active Complexones. By Yu. A. Ovchinnikov, V. T. Ivanov and A. M. Shkrob. Pp. xii+464. (Elsevier Scientific: Amsterdam, Oxford and New York, 1974.) Dfl.135; \$51.90.

spective; it is written with the same lucidity as the original papers by the authors and their coworkers. Where appropriate they start with a description of their own work and then review related topics; this approach is successful and provides painless and excellent coverage of the literature up to and including 1972 in 1,111 references

arranged in alphabetical order with a further 212 references, with titles, included to cover a few earlier papers and most of those published in 1972 and 1973. There is a good subject index.

It is unfortunate that many diagrams are reproduced from the literature and not redrawn by the excellent hand at the Shemyakin Institute because points which were obscure in the original remain so and errors are perpetuated; for example, Figure 141 shows the incorrect, absolute configuration of monensin from a preliminary communication. The sources of these diagrams are not clearly acknowledged giving a touch of irony to the publisher's almost hysterical note for forbidding reproduction. **M. R. Truter**

Biogeography

Plant Geography. (The Field of Geography) By Martin C. Kellman. Pp. xv+135+12 plates. (Methuen: London, January 1975.) £3.20 boards; £1.50 paper.

Terrestrial Environments. (Biology and Environments.) By J. L. Cloudsley-Thompson. Pp. 253. (Croom Helm: London, March 1975.) £5.95 boards; £3.25 paper.

THESE two books represent very different approaches to the task of conveying to the student in a concise form some elementary introduction to the study of biogeography. When confronted with this task, an author may present an account of general principles, illustrated by carefully chosen examples, or he may prefer to document as much factual information as possible and then attempt to stimulate the student into the formulation of general concepts for himself. Both approaches have their dangers; the former may result in an unbalanced coverage, whereas the latter may be indigestible.

Kellman's book has an honest, if traditional, title. It describes and illustrates what the author regards as the more important principles of plant geography. He begins, very sensibly, with a con-

sideration of those aspects of the environment which influence the geographical distribution of a species both at the continental and at the habitat and microhabitat level. The importance of scale in plant geographical study is thus emphasised very effectively. Plant mobility is then considered very briefly, but could have been more profitably fused with a later chapter on terrestrial vegetation history, which protrudes rather prominently from a very mixed section labelled 'Other themes in plant geography'.

Another chapter within the 'Other themes' section covers the ecosystem concept. It is very difficult for biogeography texts to avoid such a chapter in these days as systems analysis, along with multivariate statistical analyses, are currently regarded as a panacea by many biogeographers. I do feel, however, that the relevance of the concept to plant geographical studies could be conveyed more convincingly, perhaps in connection with human management of plant species and vegetation in general. A large, central section of the book deals with vegetation analysis from a practical point of view and is concerned chiefly with methods of the description of vegetation at the habitat level. Many books already exist which cover this subject in far greater detail.

Plant Geography is one of a series of university level geography texts which are designed to introduce modern developments in geographical studies and which market at a low price. Judging the book in this light and regarding it as lead towards more detailed and specialised literature, it can be considered a successful enterprise.

Cloudsley-Thompson's latest book illustrates the other approach to the transmission of biogeographical information. Its title, *Terrestrial Environments*, is trendy but misleading. It is a book about animal geography, so why not entitle it accordingly? The basic approach is encyclopaedic; the author deals with each of the major biomes in turn and provides an anecdotal, annotated list of the more interesting animals occurring within them, concentrating upon the vertebrates. Vegetation, particularly its structural aspects, is dealt with briefly and mainly from the point of view of climatic adaptation and its potential as a source of animal microhabitats.

As a botanist, I find much of the information fresh and interesting, but I wonder whether the author falls between two stools. The information-packed nature of the text, together with the fair sprinkling of unexplained technical jargon, make difficult reading. The information given is often lacking in depth and, in spite of the very extensive bibliography, is likely to leave the enquiring student somewhat dissatisfied.

Given patience and a good library for supplementary reading, however, the book could be useful. The final chapters, on such biogeographical/ecological principles as successional developments of habitats and population regulation, do not deal at all adequately with these subjects. If the author's hope is that the student who has assimilated the text will be in a position to formulate his own concepts, I fear that I disagree with him. However educationally improper it may seem, I feel that the average undergraduate needs to be fed principle as well as fact, and that factual information needs careful selection and predigestion. The practical limitations of teaching time usually force such an attitude upon us. On the whole my sympathy lies with Kellman's approach.

Peter D. Moore

Broad telescopic view

The Astronomical Telescope. By Boris V. Barlow. Pp. viii+213. (Wykeham: London and Winchester, January 1975.) £3.25 cloth; £2.50 paper.

THE large modern telescope is as much a product of engineering as optical science, so it is fitting that this excellent new book on telescopes has been written by B. V. Barlow, a mechanical engineer specialising in design specification. It has been written with the science student at universities and polytechnics in mind but it is an ideal introduction for any astronomer interested in the design and capabilities of optical telescopes. After an introduction to the history of the telescope and basic concepts in optics, image formation, interference and aberrations, the chapters deal with eyepieces, seeing and instrumental losses; telescope engineering, mirror production, mountings, tube and cell deflections and axis bearings; drive, time-keeping and computer control; ancillary equipment, spectrographs, image slicers, interferometers, photometers and bolometers; site selection and observatory buildings. There is a review of large telescopes, including the 200-inch Palomar, the Isaac Newton, the 60-inch infrared flux collector at Tenerife the Anglo-Australian, The AURA and the 6-m USSR. Extraterrestrial telescopes are also considered and Stratoscopes I and II, ESRO's ultraviolet telescope, and the NASA large space telescope are discussed. The book ends with a chapter on new developments in the use of telescopes. It is well indexed with a useful glossary and reference section and is profusely illustrated with photographs and line drawing.

The astronomy community has needed a book like this for a long time and Mr Barlow is to be congratulated on his excellent achievement.

David W. Hughes

BOOKS

ON PURE AND APPLIED SCIENCE

Books reviewed or mentioned in this journal are available from stock.

Catalogues on application.

Please state interests.

SCIENTIFIC LIBRARY

ANNUAL SUBSCRIPTION from £4.00

Reduced rates for multiple subscriptions

Available in U.K. only

Prospectus free on request

H. K. LEWIS & Co. Ltd.

LONDON: 136 GOWER STREET,
WC1E 6BS

Telephone: 01-387 4282

Petroleum geology

Introduction to Petroleum Geology. By G. D. Hobson and E. N. Tiratsoo. Pp. viii+300. (Scientific Press: Beaconsfield, March 1975.) £10.50; \$28.50.

OVER the past generation the greater number of textbooks on petroleum geology has originated in the USA, and the production of a well compiled and authoritative work by British authors is particularly to be welcomed at the time when the subject has achieved such a degree of national importance in Britain. Both authors have had long experience in this field.—G. D. Hobson on the academic and research side, E. N. Tiratsoo as a consultant with close involvement in the Middle East and Caribbean—and their combined expertise has been well applied.

The book covers the whole range of petroleum geology within the realms of exploration and production techniques, from the origin, accumulation and migration of petroleum, through exploration methods, formation logging and evaluation, the development problems, reserves assessment and—briefly—world-wide resources. It demonstrates a degree of assimilation of literature in this sphere which very few professionals would be able to match, and although the book is rather modestly stated to be “written to meet the requirements of students” there must be very few active geologists who would not find new and unfamiliar facts in its pages.

Although for the most part the book is adequately and clearly illustrated, it is unfortunate that a few of the diagrams (possibly reproduced in monochrome from coloured originals) are only legible with difficulty, and this might be given attention when a new edition is required.

Inevitably, some of the subjects described are controversial, but in such cases the authors have taken care to express both points of view, usually without taking sides.

There are a few points of special criticism: the discussion of map scales (p.135) shows a surprising absence of reference to the now almost universal use of metric values, the recommendation of mapping on 3-inches to a mile for example, is a remarkable idiosyncrasy. Some miscellaneous data are irrelevant or unnecessary—such as the different values of the gal at the poles and equator, (p.152) and the description of plane table equipment (p.137)—but, overall, the selection of information is appropriate. One may hope that in a later edition the section on seismic surveys might be expanded, but the book gives an adequate if rather short summary.

Hobson and Tiratsoo have produced a volume which should have a place on

the shelf of all serious petroleum geologists and others concerned with the technical problems of exploration and development of hydrocarbon resources; it will serve both as a very effective conspectus and as a guide to the extensive literature. **P. E. Kent**

Scattered electrons

High Energy Electron Scattering. (American Chemical Society Monograph.) By R. A. Bonham and M. Fink. Pp. vi+311. (Van Nostrand Reinhold: New York and London, June 1974.) £12.25.

THE content of this book is not as wide ranging as its title implies: the authors state in Chapter One that their interest is solely with high energy electron scattering from gaseous targets. Nevertheless, it does provide a comprehensive and lucid account of that branch of the subject, with emphasis given to the measurement of charge and momentum distributions in atoms and molecules.

The opening chapters contain a standard treatment of the relevant scattering theory and a review of theoretical methods of calculating atomic and molecular wave functions. Details are given of how elastic and total scattered electron intensity measurements may be used to obtain information on the radial distribution function and electron-pair correlation function of atoms; the experimental observations of electron correlation effects in atoms are discussed at length. The book continues with an account of the methods available for measuring the one and two-electron density function of molecules, and the results obtained from a number of molecules serve as useful illustrations. The use of intensity data to determine the three-dimensional one-electron density function is also discussed.

Consideration is given to the possibility of determining the momentum density distribution of atoms and molecules by measurement of the inelastically scattered electrons at large scattering angles from ionising collisions. That is followed by a section on high resolution, high energy impact spectroscopy with a review of the experimental results obtained. Finally, the book describes in detail the various experimental techniques used, with separate chapters on electron gun design, velocity analysers, electron detection devices, and atomic and molecular jets.

Most of the material presented is readily available elsewhere but it is useful to have the information collected in a single volume. Being completely self-contained, the book provides an appropriate balance between theory, experimental methods and results. **M. J. Capers**



THE EARTH'S DENSITY

K. E. BULLEN

May 1975: 448 pages: hardback:
412 10860 7: £12.90

This definitive *magnum opus* draws together the widespread strands of evidence that have led to the present knowledge of the distribution of density throughout the earth and the planets. The book is an authoritative source of information on a topic which bears on practically all current solid-Earth geophysical research. Recent developments of particular relevance to geophysical mineral exploration and an understanding of the planets are treated in considerable detail.

Two New Outline Studies in Biology

THE SELECTIVITY OF DRUGS

ADRIEN ALBERT

May 1975: 64 pages: paperback:
412 13090 4: £1.30

This book presents an overall picture of the physical basis of drug action based on three principles – favourable differences in distribution, favourable differences in biochemistry and favourable differences in cell structure – and shows how these principles control selectivity.

BIOMECHANICS

R. McNEILL ALEXANDER

May 1975: 64 pages: paperback:
412 13080 7: £1.30

This book presents a broad consideration of the application of mechanics to the study of organisms. Descriptions of biological materials, animal locomotion, human mechanics, plant mechanics and cell mechanics are all included.

Leaflets on Earth Sciences books and on the Outline Studies in Biology Series are available from the publisher on request.

CHAPMAN & HALL

11 New Fetter Lane,
London EC4P 4EE



announcements

Awards

The Gravity Research Foundation (Gloucester, Massachusetts) has given the **Non-linear Graviton Award** to **Roger Penrose**.

The Chemical Society has announced the following honorary fellowships: **F. Lynen**, **L. Malatesta**, **Sir David Martin**, CBE and **G. Ourisson**.

F. Bronner has been awarded the **André Lichtwitz Prize** for his work on calcium transport.

R. P. Bodnaryk is the first winner of the **C. Gordon Hewitt Award** (offered annually by the Entomological Society of Canada for exceptional accomplishment related to the study of insects by a young scientist). The Society's **Gold Medal** has been awarded to **G. G. E. Scudder** (systematics, morphology and distribution of Lygaeidae).

Appointment

The Institution of Electrical Engineers has announced that its next president will be **R. J. Clayton**.

Miscellaneous

Lichtwitz prize. This prize is meant to reward French or foreign scientists who have distinguished themselves in research on calcium and phosphorus metabolism, whether in the clinical or more fundamental fields. Conditions of entry for the 1975 prize (applications by June 30) may be obtained from: Director of INSERM, c/o Dr L. Bonnot, 101 rue de Tolbiac, 75645 Paris CEDEX 13, France.

Pathology award. The Fondation de Physiopathologie Professeur Lucien Dautrebande will award a prize (500,000 Belgian francs) during 1976 for work on human or animal clinical physiopathology, preferably having therapeutic implications. For further information: 35 chaussée de Liège (5200), Huy, Belgium (before December, 1975).

Endangered flora. Smithsonian Institution's *Report on Endangered and Threatened Plant Species of the United States* are available, on written request, from: Endangered Flora Project, Department of Botany, Smithsonian Institution, Washington, DC 20560.

Person to Person

Philadelphia - Sussex/London/Cambridge. Accommodation (centre of city, five bedrooms with usual American conveniences) for exchange with suitable study accommodation near University of Sussex, or in London, or easy access from London to Cambridge. Academic couple, three school-age daughters. Approximate dates: September 1, 1975 to May 15, 1976 (B. C. Griffith, Library of Science, Drexel University, Philadelphia, Pennsylvania 19104).

London accommodation. Wanted August (and possibly September) by visiting research biologist and family. Three bedroom flat or house within reasonable distance of University College, London. (Dr D. Whittingham, MRC Mammalian Development Unit, Wolfson House, 4 Stephenson Way, London NW1 2HE, UK; 01-387 7050, ext. 728).

London-Charleston. Accommodation required within reasonable distance of Medical University of South Carolina, Charleston from January, 1976 to January, 1977. Can exchange for modern 3 bedroomed house (central heating) in London (Dr R. M. Galbraith, Liver Unit, King's College Hospital, Denmark Hill, London SE5 9RS, UK).

Coleus blumei. Biologist interested in using this species for demonstration purposes and as object for aesthetic appreciation wishes to receive any information (reprints or correspondence) about colour and morphological mutations. Possible exchange of genetic materials (Dr R. E. Gross, 74 Tübingen, Albrechtstrasse 9, West Germany).

Griffith observer magazine. For January 1975. Copy wanted. If desired, would swap for *Observer* equivalent (Lillian Thompson, Flat 2, 14 College Terrace, Brighton BN 2 2EE, Sussex, UK).

There will be no charge for this service. Send items (not more than 60 words) to Robert Vickers at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

Reports and publications

Great Britain

The Chemical Industry and the EEC. (A statement by the Trade Affairs Board of the Chemical Industries Association.) Pp. 4. (London: Chemical Industries Association, 1975.) [203]
 Flora of Tropical East Africa. Edited by R. M. Polhill. Bixaceae. By D. M. Bridson, Pp. 3. (London: Crown Agents for Oversea Governments and Administrations, 1975.) 15p. [213]
 Imperial Cancer Research Fund. Annual Report and Accounts 1974. Pp. 41. Scientific Report 1974. Pp. 165. (London: Imperial Cancer Research Fund, 1975.) [213]
 Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 278, No. 1279: Second-order Linear Differential Equations with Two Turning Points. By F. W. J. Olver. Legendre Functions with Both Parameters Large. By F. W. J. Olver. Pp. 137-185. (London: The Royal Society, 1975.) UK £2.05; Overseas £2.15. [213]
 Energy Economy in Industry: 150 Fuel Saving Ideas. By Nigel Gwyther. Pp. 38. (Melton Mowbray: T.G.W. Industrial and Research Promotions, Ltd., 29 Dalby Road, 1975.) 80p. [243]
 Agricultural Development and Advisory Service. Science Arm—Annual Report 1973. Pp. xxxviii + 257. (London: HMSO, 1975.) £5.50 net. [253]
 Department of the Environment—Central Unit on Environmental Pollution. Pollution Paper No. 4: Controlling Pollution. Pp. iii + 31. (London: HMSO, 1975.) 42p net. [263]
 Archaeological Investigations in New Palace Yard 1972-74. Pp. 16. (London: Department of the Environment, 1975.) [273]
 Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 270, No. 904: Natural Regeneration of *Tilia cordata* in Relation to Forest-Structure in the Forest of Biatowieza, Poland. By C. D. Pigott. Pp. 151-179. (London: Royal Society of London, 1975.) [273]

Other countries

Energy Communications. Vol. 1, No. 1, 1975. Edited by R. N. Maddox. Pp. 1-110. Subscription rate for Vol. 1 (1975), 6 issues: \$40 prepaid. Postage outside USA \$6.30 per volume. (New York: Marcel Dekker, Inc., 1975.) [243]
 Institutt for Atomenergi, Kjeller, Norway. Kjeller Report 151: Multec Version 1.0. A Program for Multivariable Estimation and Control of Dynamic Systems—Users Manual. By Arild Ek and Erik Gran. Pp. 44. (Kjeller: Institutt for Atomenergi, 1975.) [243]
 Science Council of Canada. Report No. 23: Canada's Energy Opportunities. Pp. 135. \$3.30. Summary. Pp. 28. (Ottawa: Information Canada, 1975.) [253]
 World Health Organization. Guide to Simple Sanitary Measures for the Control of Enteric Diseases. By S. Rajagopalan. With a section of Food Sanitation by M. A. Shiffman. Pp. 103. (Geneva: WHO; London: HMSO, 1974.) Sw.fr.32. [253]
 Publications of the Dominion Astrophysical Observatory, Victoria, BC. Vol. XIV, No. 11: A Simultaneous Two-Channel System for Lunar Occultation Observations. By C. L. Morbey and J. M. Fletcher. Pp. 271-281. Vol. XIV, No. 12: Spectroscopic Observations of Stars in HII Regions. By David Crampton and W. A. Fisher. Pp. 283-304. (Victoria, BC: Dominion Astrophysical Observatory, 1974.) [253]
 World Health Organization. Food Additives Series No. 6: Toxicological Evaluation of some Food Colours, Enzymes, Flavour Enhancers, Thickening Agents, and Certain other Food Additives. Pp. 204. (Geneva: WHO; London: HMSO, 1975.) [273]
 Essai d'Explication Optique des Points Chauds de la Terre—Conséquences. Par Georges Dubourdieu. Pp. 55. (Meudon: Collège de France, Laboratoire de Géologie, 1975.) [273]
 Annual Report of the Zoological Institute, Faculty of Science, University of Tokyo, 1974. Pp. 27. (Tokyo: The University, 1975.) [14]
 Glossary of Soil Science Terms. Pp. 34. (Madison, Wisconsin: Soil Science Society of America, 677 South Segoe Road, 1975.) \$1. [14]
 World Health Organization. Technical Report Series, No. 559: New Approaches in Health Statistics—Report of the Second International Conference of National Committees on Vital and Health Statistics. Pp. 40. Sw.fr.5. International Histological Classification of Tumours No. 11: Histological Typing of Thyroid Tumours. By Chr. Hedinger, in collaboration with L. H. Sobin. Pp. 28 + 46 figures. Sw.fr.28. (Geneva: WHO; London: HMSO, 1974.) [24]
 Guides and Criteria for Recreational Quality of Beaches and Coastal Waters. (Report on a Working Group convened by the Regional Office for Europe of the World Health Organization. Bilthoven, 28 October-1 November 1974.) Pp. iii + 31. (Copenhagen: Regional Office for Europe, World Health Organization, 1975.) [34]